ANALYSIS OF PROGRAM ACTIVITIES
NATIONAL INSTITUTES OF HEALTH
1955

NATIONAL INSTITUTE OF
ALLERGY AND INFECTIOUS DISEASES

NATIONAL INSTITUTE OF DENTAL RESEARCH

NATIONAL INSTITUTES OF HEALTH
PUBLIC HEALTH SERVICE
U. S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

U.S. NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES
" Report of program activities.
SUPERSEDED

THE NATIONAL MICROBIOLOGICAL INSTITUTE

DECEMBER 29, 1955

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Analysis of NIH Program Activities

January-December 1955

National Microbiological Institute
Laboratory Research

While fundamental problems in microbiology have continued to receive individual attention from investigators in the basic laboratories, a different type of approach has characterized some of the major efforts of the Institute during 1955. Scientists representing several disciplines have combined forces in order to attack on a broad front such major problems as respiratory diseases, minor illnesses in a rural community, and poliomyelitis.

Studies on the adenoidal-pharyngeal-conjunctival (APC) virus have been continued. Definite evidence was obtained that monovalent vaccine affords protection against experimental conjunctival infection in human volunteers. This investigation is being expanded to include the use of polyvalent strains in larger population groups in a field trial designed to evaluate the effectiveness of the vaccine in preventing acute respiratory disease. Other related studies have clarified the role of the APC agents as a cause of disease and have resulted in the identification of 12 immunological human types. In collaboration with scientists of the National Cancer Institute studies have been continued on the oncolytic effects of these viruses on one type of human cancer.

During the year, major changes in the research program of the Rocky Mountain Laboratory were instituted. These were in a large measure concerned with the development of a large-scale study of minor illnesses in a rural community. The objectives of this Community Health Project were to discover the major causes of febrile illnesses in such a community, to determine the type of illness associated with specific agents, to develop diagnostic methods, to elaborate the epidemiology of these illnesses, and to discover methods for the specific control and prevention of such diseases. While somewhat similar studies are being carried out by other groups in urban centers, institutions of various types, and in selected groups in heavily populated areas, the results obtained in these situations may not be applicable to a semi-isolated rural area. The community under study presents several unique opportunities which permit more precise studies under better controlled conditions than those afforded by urban populations where the incidence of immunity is high and the tracing of an infection chain very difficult. The initial organization and facilities were set up in time to take full advantage of an outbreak of poliomyelitis in the Pribilof Islands and of the unusual situation developing in Idaho during the poliomyelitis vaccination program. The latter study, while quite productive, markedly curtailed the activities originally planned for this project. However, results of preliminary studies in this area showed that 50 per cent of school children had antibodies against APC virus and that strain A8 Coxsackie virus was epidemic during the year with a marked correlation between the presence of these agents and the occurrence of diarrheal disease. A study of the sewers in the community indicated that there must be a widespread occurrence of a given virus in the population some weeks before actual cases of virus disease appear. This finding could be of considerable epidemiological significance.

In studies on basic immunology, increasing emphasis has been directed toward the characterization and purification of antigenic material. By applying the concept that protective antibodies should be present within or on the cell wall of an organism, it has been possible to obtain fractions which at least are morphologically pure and which have increased antigenicity and decreased toxicity. That this concept of surface antigens may be generally applicable appears to be substantiated by the fact that such immunizing products have now been obtained from a fungus and two genera of bacteria.

In the field of insect-borne diseases, studies have been directed primarily toward Colorado tick fever and the viral encephalitides. The former is a significant disease among persons engaged in rural occupations and those employed in the tourist industry and has been found to produce serious encephalitis in children. The relationship of arthropod vectors and possible animal hosts requires further study before the epidemiology of the disease can be understood and effective control measures developed. Likewise, the control of western equine encephalitis awaits the determination of whether the endemic and epidemic incidence of this disease is related to over-wintering of infected arthropods or to the introduction of infected animal hosts. Studies on these problems are continuing.

In anticipation of the establishment of a germ-free animal program at Bethesda, the cooperative study carried on by an Institute scientist at the University of Notre Dame has been continued. Further investigations on the effect of bacteria upon the disease producing potential of *Entamoeba histolytica* indicates that heat-killed bacteria contain some substance which is capable of triggering the pathogenic capability of the amoeba.

During the course of investigations on the chemotherapy of parasitic diseases the antibiotic, Puromycin, was found effective in limited clinical trials in eliminating all intestinal protozoan parasites from a small number of patients. Other results in experimental animals indicate that this drug may have a long residual prophylactic action against at least one of the African species of *Trypanosoma*.

A project was inaugurated on the incidence, epidemiology, diagnosis, and treatment of *Trichomonas vaginalis* infection. Current findings indicate that transmission is possible and even probable through the agency of fomites. There was no evidence of direct association between *T. tenax* of the mouth, *T. hominis* of the intestinal tract and *T. vaginalis* of the female genital tract.

As a logical conclusion to investigations carried on during previous years on schistosomiasis control, a scientist of this Institute was detailed to a cooperative pilot control project in Egypt sponsored by PHS, ICA, and the Egyptian Ministry of Health. Here sodium pentachlorophenate was applied to an area of 40 square miles during the year ending October 31, 1955, resulting in the complete elimination of all snails from the area. Surveys on school children within the area prior to application of the chemical indicated an incidence of 52 per cent infection which at the end of the year this incidence had declined to 44 per cent.

As a result of recent, almost incidental, findings, scientific interest in pentachlorophenol is no longer restricted to its lethal effect on snails. Laboratory studies have shown that this chemical exercises a disruptive effect on certain enzymatic activities within the individual cell. This knowledge provides a new resource for investigating the intricate and obscure life processes which take place at the cell level.

Intense interest in the use of tissue culture for the study of causative agents of infectious diseases has characterized many of the activities of the past year. The increasing number of techniques and expanding knowledge of tissue culture procedures, in the development of which the National Microbiological Institute has been in the forefront, have led to a number of significant advances.

The demonstration that monkey kidney tissue cultures are superior to embryonated eggs for the primary isolation of influenza B virus was an important result of the influenza studies.

Advances have been made in the determination of metabolic activities and nutritional requirements of normal and malignant cells both uninfected and infected with viruses. Amino acid, vitamin, and electrolyte requirements have been determined. These have lead to practical improvements in the methods for maintaining and growing both normal and malignant cells in tissue culture and to further elucidation of the metabolic pathways of cell growth and viral synthesis. Differences have been demonstrated in the metabolism of nucleic acid components between normal and virus infected cells and it has been shown that glucose and glutamine are essential materials for the support of poliomyelitis virus in HeLa cell cultures.

Observations have been made on the morphological changes occurring in cells of virus infected tissue cultures. Results to date indicate that the type of changes occurring during degeneration and dissolution of the cell is influenced by the type of cell attacked and by the particular virus being grown.

The realignment of research projects in keeping with current needs and developments has continued. In 1955 a training program for senior investigators in this Institute was established. As a result staff members have worked in the laboratories of Dr. Paul Melnick at Yale University, Dr. John F. Enders, Harvard University, and Dr. A. M. Pappenheimer, Jr., New York University. The Institute's research program has been materially strengthened as a result of the techniques and new approaches thus acquired.

Analysis of NIH Program Activities

January-December 1955

National Microbiological Institute
Clinical Investigations

The over-all research program of the Institute has been strengthened materially by the availability of clinical facilities, and having a group of clinically oriented scientists on the staff has permitted the undertaking of such basic studies as those on agammaglobulinemia and lymph node grafting.

The staff engaged in medical care and clinical research has been increased during this year by the addition of six medical officers, two of whom are pediatricians. This additional medical personnel has provided more adequately the necessary breadth and depth of medical coverage for the clinical operations.

The supportive biochemical area has been strengthened by the addition of a biochemist and an immunochemist trained in the disciplines of medicine and physical chemistry. This development has allowed the undertaking of research in the field of immunochemistry which will serve as a nucleus around which to develop a research program in the field of allergic diseases and has strengthened the projects on fundamental studies of tissue cultures and host-parasite relationships at the cellular level. The services of a full-time bacteriologist and staff now permit increased emphasis on those projects demanding bacteriological competence and aid in their execution.

The admission this year of poliomyelitis patients, who require a great amount of nursing care, and the fact that many patients whom we study are children or seriously ill individuals have influenced the percentage of bed occupancy. Under such circumstances, adequate nursing care can be provided only by reducing the census. Although fully utilizing the available nursing coverage throughout the entire year, the percentage of bed occupancy during the year was 57 per cent. This necessary reduction in the number of patients has, of course, slowed the research program in certain areas.

General Trends

With the over-all increase in personnel and the strengthening of the clinical supportive services, research productivity has increased and there has been a general trend toward more basic investigations. More strictly clinical studies continue but are supplemented and supported by basic studies. Some of these basic investigations, e.g., the studies on serum proteins, cut across several project lines while another has resulted in the defining of a serum fraction essential to the growth of cells in tissue culture.

In the infectious disease projects, there continues a strong emphasis on viral diseases. This has included the admission of patients with poliomyelitis and aseptic meningitis. It is presumed that this emphasis on viral diseases will continue. Due to the availability of a number of suitable and unique cases, increased effort has been expended on the project on systemic fungal diseases.

There has been further development in cooperative research with the non-clinical laboratories within the Institute. In addition to studies previously initiated, there are two noteworthy additions. An extensive study on trichomoniasis has been undertaken, utilizing material from cytological surveys. Another cooperative enterprise developed in the field of poliomyelitis and aseptic meningitis. This worthwhile study was possible only on such a collaborative basis. Particular note should also be given to the extensive and continuing cooperative research on the systemic mycoses. Collaborative studies with other Institutes are being conducted on pulmonary physiology, metabolism in hepatitis, nephritis, infections in leukemia, granulomatous pulmonary diseases, brucellosis, sarcoidosis and toxoplasmosis.

Comments on Specific Projects

Extensive investigations have been conducted on patients with agammaglobulinemia. The metabolism of administered extraneous gamma globulin has been studied and results strongly suggest that different antibodies are metabolized at different rates in the same individual. A successful lymph node transplantation experiment was performed; nodes from a human donor implanted into an agammaglobulinemic patient remained functional for approximately one hundred and sixty days. It was possible, from these studies, to estimate for the first time the rate of protein synthesis in vivo in man per unit of tissue per unit of time.

Data on twenty-four established and thoroughly studied patients with sarcoidosis have been accumulated. One basic approach to the study of this disease has been the use of tissue explants in culture for studies of reactivity to various substances. Insofar as is known, no other center is employing this most promising approach. Differences have been noted in growth rates, cell types, and cytopathogenic effect of tuberculin, etc., between sarcoid tissue, tuberculin sensitive and insensitive tissue, histoplasmin sensitive and normal tissue. The studies are sufficiently far along to warrant the expectation that they will yield basic information regarding cellular immunology and reactivity.

During studies on respiratory viral diseases it was found that from nineteen children who were currently asymptomatic but who were subjected to tonsillectomy and adenoidectomy, APC viruses were recovered in fifteen instances and one unidentified virus in a further instance. Notable progress has been made in the further delineation of Coxsackie B virus infections and in the clinical differentiation of herpetic gingivostomatitis and herpangina.

In the study on microbiologic flora major emphasis was placed on a uniform group pf patients who have fibrocystic disease and who harbor continually various strains of staphlococci. These patients, who usually die as a direct result of chronic and continuing infections, predominantly due to the staphlococcus, afford a unique opportunity for research. Several studies are in progress on this group of patients relative to immunological defense mechanisms, persistence of infection, etc. Currently, it appears that these patients harbor each his own strain of staphlococcus continuously, rather than experience repeated new infections.

In the latter part of the year, a request was received for advice and help in the paratyphoid B epidemic occurring in Lancaster, Pennsylvania. A partial study in the clinical area was undertaken and is still continuing.

Patients referred with the diagnosis or suspicion of parasitic disease have been accepted as usual. During the last year, we have had access to one large survey group being studied for another purpose and have instituted our own survey on foreign students attending Howard University. The study of one case of laboratory acquired toxoplasmosis has yielded new information on incubation period, parasitemia, immunological responses, and therapy.

Analysis of NIH Program Activities

January-December 1955

National Microbiological Institute
Extramural Activities

The National Microbiological Institute supports two extramural activities (1) grants-in-aid for research and (2) fellowships. At the close of the calendar year 1955, there were 212 research grants ($2,222,854) and 34 fellowships in active status.

Program Trends and Developments

Research projects supported by grants encompassed 17 major areas of microbiology: antibiotics (4), arthropods (24), bacteriology (46), biochemistry (15), rapid identification of microorganisms (3), epidemiology (1), mycology (18), genetics (1), helminthology (27), immunology (9), physiology (2), protozoology (27), public health and sanitation (6), rickettsiology (4), spiorchete studies (2), taxonomy (1), and virology (35). Both study sections and the National Advisory Health Council continually expressed their interest in furthering specific investigations in areas of promise but were hampered in the fulfillment of objectives by the shortage in funds.

It is particularly noteworthy that in virology there are indications of increasing research activity in salivary gland viruses, variola, dysentery, rubella, herpes, infectious mononucleosis, and encephalitis. These diseases, most common among children, cost millions of dollars each year and are the source of suffering for the child and of considerable anxiety to the parent. The interest of investigators in these areas, and in general basic immunology, is noteworthy because of the long-recognized need for emphasis in pediatrics.

In parasitology there are evidences of increasing activity in the ubiquitous helminth diseases: hookworm, roundworm, pinworm, whipworm, and tapeworm. Since children are especially prone to infestations, studies in this field are of direct significance to the pediatrician. In the general area of parasitology and tropical medicine, 78 grants, including projects on helminthology, arthropods, and protozoal diseases, are presently active: among these studies are investigations on filariasis, schistosomiasis, and trichinosis. Although the National Microbiological Institute was unable to finance the parasitology training program recommended by the Study Section and Council, the action of these panels was highly instrumental in the awarding of a two-year grant of $80,000 by the China Medical Board to Louisiana State University for the purpose of administering such a program in Costa Rica, Puerto Rico, and Guatemala. These traineeships will number approximately 20 each year for the two-year period which began in the summer of 1955.

Accomplishment Highlights

Significant contributions, both of a practical and a fundamental nature, have been made by grantees of this Institute.

A second strain of the human salivary gland virus appears to have been isolated by investigators at Washington University from the organs of an infant dying of the congenital form of cytomegalic inclusion disease. This new agent produces in cultures of human uterine tissue cytopathic changes which appear identical with those produced by the virus originally isolated from a human salivary gland.

Recent investigations by scientists at the Medical College of South Carolina indicate that mice inoculated with *Hemophilus pertussis* vaccine are particularly vulnerable to active or passive anaphylaxis. Vaccinated mice were found to be 268 times more sensitive to the lethal effect of histamine than were the uninoculated control animals. Preliminary results indicate that this anaphylactic reaction in mice is inhibited by Neoantergan, one of the newer antihistaminics.

Clinical trial of an experimental vaccine against one type of common respiratory illness was reported by scientists of this Institute working in cooperation with a Johns Hopkins investigator whose studies are supported by an NMI grant-in-aid. Experiments conducted on human volunteers indicate that the vaccine provides substantial protection against one of the ten types of APC (adenoidal, pharyngeal, conjunctival) viruses.

Chemical fractionation of pathogenic rickettsiae, carried out by a grantee at Harvard University resulted in the isolation of serologically active erythrocyte sensitizing substances. These highly sensitive substances proved of value in the serological diagnosis of epidemic and murine typhus as well as Rocky Mountain spotted fever and rickettsialpox. An incidental finding of this same group resulted in the development of a simple method for the continuous subcultivation of epithelial cells from normal human liver, conjunctiva, kidney and appendix. Studies are now being conducted on the use of hepatic cells for the isolation of infectious hepatitis virus and conjunctival cells for propagation of viruses from the human eye.

In past years tuberculous meningitis, a particular threat to children, meant either death or hopeless crippling. This outlook now appears to be changing. From New York's Bellevue Hospital an investigator reports preliminary results indicating that isoniazid is effective in preventing the development of meningitis in children hospitalized for primary pulmonary tuberculosis.

Two investigators at the University of California are attempting to isolate and identify a possible viral agent in respiratory tract infections in man. They have accumulated evidence suggesting a possible direct relationship between canine distemper, which is the commonest viral respiratory disease of dogs, and a respiratory tract infection of humans.

R. P. C. - 1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

1. National Microbiological Institute
INSTITUTE

2. Office of the Director
LABORATORY OR BRANCH

3. None
SECTION

4. Bethesda, Maryland
LOCATION

5. NMI - 1
SERIAL NO.

6. Office of the Director
PROJECT TITLE

7. Dr. Victor H. Haas
PRINCIPAL INVESTIGATOR

8. OTHER INVESTIGATORS:

None

9. PROJECT DESCRIPTION

Objectives and Accomplishment

(1) The program objectives of the Institute and its position in the progress and conduct of basic research have been affected by several important developments during the current year.

Activities related to the control and licensing of biologics were transferred from the administrative jurisdiction of the National Microbiological Institute. This action, which terminates a traditional relationship of long standing, serves not only to strengthen the biologics control program but serves to delineate and point up the fundamental research responsibility and interest of the Institute.

Of major importance during this year has been the recognition of need for intensified research in the field of Allergies. Although only limited funds are presently available to implement this work, administrative review of a number of the Institute's programs is underway which, it is believed, will result in the redirection of some research interest and a substantial start in a new program of allergy studies.

A start has been made this year through the commitment of space and funds toward the establishment of a Germ Free facility. This program as it develops will attract particular interest since it will permit redirection of several parasitic disease studies into a new although related field.

Objectives and Accomplishments (Continued)

(2) To coordinate the activities of the Institute with those of the National Institutes of Health, the Public Health Service, and where appropriate, with other research agencies of the Federal Government.

In addition to the appropriate and normal adjustment to administrative and management developments affecting all of NIH, several of the more important specific cooperative research projects may be of interest: (1) The joint research of the Laboratory of Tropical Diseases and the Institute of Neurological Diseases and Blindness on toxoplasmic eye diseases.

(2) Virus-cancer cell studies in which the Cancer Institute and NMI are jointly working.

(3) Cancer Chemotherapy study on which the Laboratory of Infectious Diseases is collaborating.

(3) To insure that information on the research activities of the Institute is adequately disseminated to other scientific organizations, to Congress, and to the general public.

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December 1955

Budget Data Sheet

10.

11. BUDGET DATA:

ESTIMATED	BUDGETED POSITIONS			MAN YEARS			PATIENT
EXPENDITURES	PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	DAYS

12. BUDGET ACTIVITY:

Research (Information Officer)
Administrative

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957:

None

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES, OR FUNDS), IDENTIFY SUCH RESEARCH:

None

Honors, Awards, and Publications Sheet

15.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

Haas, Victor H. Inhabitory effect of virus of lymphocytic choriomeningitis on course of leukemia in guinea pigs. Reprinted from Federation Proceedings Vol. 14, No. 1, March 1955.

Haas, Victor H. Unsolved Problems in Microbiology. Reprinted from Military Medicine, Vol. 116, No. 2, February, 1955.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

None.

R. P. C. - 1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

1. National Microbiological Institute
INSTITUTE

2. Office of the Director
LABORATORY OR BRANCH

3. None
SECTION

4. Bethesda, Maryland
LOCATION

5. NMI - 2
SERIAL NO.

6. Research Auxiliary Section
PROJECT TITLE

7. Kenneth H. Brown
PRINCIPAL INVESTIGATOR

8. Earl Dillehay
IN CHARGE

9. PROJECT DESCRIPTION

Objectives and Accomplishments:

The purpose of the Research Auxiliary Section is to provide central services which cannot be effectively performed by the individual laboratories. The work of this group includes supplying the laboratories with animal food, bedding, dry ice; cleaning animal rooms and cages; operating the building incinerators; providing central cage sterilization; continuing over weekends the feeding and observation of animals; collect dirty glassware from the laboratories, replacing it with sterile glass supply, maintained in central self-service,glass stock room.

R.P.C. - 2
December 1955

Analysis of NIH Program Activities SERIAL NO. NMI - 2

Budget Data Sheet

10.

11. BUDGET DATA:

ESTIMATED EXPENDITURES	BUDGET POSITIONS			MAN YEARS			PATIENT DAYS
	PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	

12. BUDGET ACTIVITY:

Research

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957:

None

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES, OR FUNDS), IDENTIFY SUCH RESEARCH:

None

Honors, Awards, and Publications Sheet

15.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

None

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

None

R.P.C. - 1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

1. National Microbiological Institute
2. Extramural Program

3. None
4.
5. NMI - 3
SERIAL NO.

7. Dr. Leonard Karel
PRINCIPAL INVESTIGATOR

8. None

9. PROJECT DESCRIPTION

I. RESEARCH GRANTS REVIEWED DURING CALENDAR YEAR 1955
(Fiscal Year 1956 Grants Appropriation - $2,110,000)

	REQUESTED		APPROVED	
February 1955 (F.Y. 1955)	No.	Amount	No.	Amount
New	69	$668,884	51	$480,232
Continuations	35	301,756	34	280,646
Total	104	$970,640	85	$760,878
June 1955 (F.Y. 1956)				
New	82	$ 839,822	62	$ 595,852
Continuations	117	1,313,061	112	1,269,901
Total	199	$2,152,883	174	$1,865,753
October 1955 (F.Y. 1956)				
New	50	$ 512,367	33	$314,265
Continuations	76	700,684	69	598,497
Total	126	$1,213,051	102	$912,762

Note: Applications to be considered by the Study Sections and Council beginning January 1955 are not included in these figures.

Of the applications recommended for approval during the calendar year, 46 for $438,806 were transferred to other categorical programs for payment; 72 for $635,375 were inactivated, and 45 for $409,811 are being held for possible awards from fiscal 1957 funds. Thus the Institute itself was unable to award Council-approved grants to the extent of 163 for a total of $1,483,992.

PROJECT DESCRIPTION (Continued)

II. MICROBIOLOGICAL INSTITUTE FELLOWSHIPS REVIEWED DURING CALENDAR YEAR 1955:

Fellowship applications considered by the National Microbiological Institute Board	155
Fellowship applications approved by the National Microbiological Institute Board	145
Fellowships awarded by the National Microbiological Institute	34
Fellowship applications approved by the National Microbiological Institute Board and paid from funds from other Institutes	50
National Microbiological Institute applications approved but remaining unpaid for lack of funds	61

Fellowships awarded by the National Microbiological Institute were spread through the following areas of microbiology: antibiotics (1), arthropods (2), bacteriology (5), biochemistry (6), helminthology (3), immunology (8), physiology (1), protozoology (3), public health (1), rickettsiology (2), and virology (2).

SERIAL NO. NMI - 3

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December 1955

Analysis of NIH Program Activities

Budget Data Sheet

10.

11.

12. BUDGET ACTIVITY:

REVIEW & APPROVAL

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 OR 1957:

Division of Research Grants

14. None

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December 1955

Analysis of NIH Program Activities

Honors, Awards, and Publications Sheet

15.

16. None

17. None

R. P. C. - 1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

1. National Microbiological Institute
INSTITUTE

2. Laboratory of Clinical Investigation
LABORATORY OR BRANCH

3. None
SECTION

4. Bethesda, Maryland
LOCATION

5. NMI- 4C
SERIAL No.

6. Office of the Chief
PROJECT TITLE

7. Dr. Norman B. McCullough
PRINCIPAL INVESTIGATOR

8. None
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives:

To develop, direct and coordinate the program of the Laboratory, as defined in the individual research projects; where necessary, to re-direct individual projects to meet current needs and advances in the field; to align clinical projects with patient availability; to provide an unexcelled standard of patient care for patients utilized in research.

Methods Employed:

Organize available staff and recruit qualified personnel, both professional and sub-professional, to develop the program and carry out its aims; close liaison with area medical societies, institutions and individuals for referral of patients whose diagnoses fall within the active or proposed disease research categories; the highest standards of patient care are maintained by selection of qualified physicians; the development of necessary policies and continued close and direct supervision of patient care activities; professional consultant services to other institutes in the area of infectious diseases; continued guidance of research projects undertaken by younger investigators; maintenance of staff morale.

R. P. C. - 2 Analysis of NIH Program Activities SERIAL NO. NMI-4C
December 1955

Budget Data Sheet

10.

11. BUDGET DATA:

ESTIMATED	BUDGETED POSITIONS			MAN YEARS			PATIENT
EXPENDITURES	PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	DAYS

12. BUDGET ACTIVITY:

Research

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATION PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 OR 1957:

None

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS) IDENTIFY SUCH RESEARCH:

None

Honors, Awards, and Publications Sheet

15.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

None

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

None

R. P. C. - 1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

1. National Microbiological Institute
INSTITUTE

2. Laboratory of Clinical Investigation
LABORATORY OR BRANCH

3. Biochemistry-Immunochemistry
SECTION

4. Bethesda, Maryland
LOCATION

5. NMI- 5C
SERIAL NO.

6. Immunochemistry
PROJECT TITLE

7. Dr. Sheldon Dray
PRINCIPAL INVESTIGATOR

8. None
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives:

The general objective of this project is to contribute to the understanding of immunologic and allergic phenomena in terms of physical and chemical reactions. More specifically, the following problems will be undertaken:

1. To develop methods for the quantitative determination of non-precipitating antibodies such as found in allergic diseases.

2. To extend the existing methods for the quantitative determination of the antigenic components of human tissues.

3. To develop a well-defined system, possibly by the use of tissue culture, for the study of the biosynthesis of antibodies.

4. To study the mechanism of tissue damage resulting from antigen-antibody reactions.

5. To develop better methods for the isolation and purification of the specific antigens in various pollens and microorganisms.

Methods Employed:

1. The gel diffusion method for the immunochemical analysis of antigen-antibody reactions.

2. Chemical analysis of antigen-antibody precipitates.

3. Freunds adjuvant technique for the immunization of rabbits.

4. Prausnitz-Kustner passive transfer technique for the determination of reagin.

R. P. C. - 1 Analysis of NIH Program Activities SERIAL NO. NMI- 5C
December 1955

Project Description Sheet

. Patient Material:

None

Major Findings:

New project

Significance to Microbiological Research:

Studies in immunochemistry should lead to a better understanding of the pathogenesis of allergic and infectious diseases and thereby provide a basis for better clinical management of these diseases.

Proposed Course of Project:

As described

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December 1955

Budget Data Sheet

10.

11. BUDGET DATA:

ESTIMATED	BUDGETED POSITIONS			MAN YEARS			PATIENT
EXPENDITURES	PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	DAYS

12. BUDGET ACTIVITY:

Research

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 OR 1957:

None

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS) IDENTIFY SUCH RESEARCH:

None

Honors, Awards, and Publications Sheet

15.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

None

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

None

R. P. C. - 1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

1. National Microbiological Institute
INSTITUTE

2. Laboratory of Clinical Investigation
LABORATORY OR BRANCH

3. Biochemistry
SECTION

4. Bethesda, Maryland
LOCATION

5. NMI- 6C
SERIAL NO.

6. Studies on the biochemical responses of the body to infectious disease agents.
PROJECT TITLE

7. Dr. Harry G. Steinman
PRINCIPAL INVESTIGATOR

8. Dr. Henry G. Cramblett, Dr. Lewis E. Gibson and Dr. Sheldon Dray
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives:

To study the biochemical changes that accompany infectious disease processes, individual metabolic processes will be examined.

Methods Employed:

Electrophoretic patterns of the plasma proteins of all patients on the Infectious Disease ward, are being followed at periodic intervals throughout the course of illness(to determine the patterns that obtain) and as an aid to determining the effectiveness of therapeutic measures.

B. A number of specific antibodies will be quantitatively determined in all patients throughout the course of their infection by means of new micro-techniques. It is hoped that these techniques will enable the determination of antibodies hitherto undetectable.

C. A number of biochemical studies will be made in various disease entities in order to learn something about the factors which produced the diseases as well as the effects brought about by the diseases. For example, ion transport will be investigated in children with fibrocystic disease of the pancreas because of the findings that their perspiration is usually high in sodium chloride.

D. A wide variety of chemical determinations will be made throughout the course of a number of infectious diseases. These will consist of studies on the sodium chloride content of sweat, sputum, etc., of the amino acid composition of blood; and of the metabolic fate of antibiotics and other drugs.

Project Description Sheet

Patient Material:

		No.	Average Stay Days
Admissions:	Adult males	11	2
	Adult females	13	4
	Children	0	0
Outpatients:	Number of patients	2	
	Number of visits	6	

Major Findings:

The electrophoretic patterns of a number of patients with infectious hepatitis were followed throughout the course of their illnesses. It was observed that there was significant and characteristic inversions in the quantitative relationships of the various protein components in the plasma of these patients several weeks after onset of their illnesses. These studies are being extended to other infectious diseases.

Significance to Microbiological Research:

Such biochemical studies of the effects produced by infectious diseases on the whole host should prove helpful in developing new diagnostic and therapeutic techniques.

These investigations are made possible by the availability of selected groups of patients admitted on other projects. Several of the diseases under study are of such a complex nature, i.e., fibrocystic disease of the pancreas in children, that little is known of their etiology and further advances demand more basic biochemical knowledge.

Proposed Course of Project:

Continue as described.

R. P. C. - 2 Analysis of NIH Program Activities SERIAL NO. NMI-6C
December 1955

Budget Data Sheet

10.

11. BUDGET DATA:

ESTIMATED	BUDGETED POSITIONS			MAN YEARS			PATIENT
EXPENDITURES	PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	DAYS

12. BUDGET ACTIVITY:

Research

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATION PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 OR 1957:

None

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

None

R. P. C. - 3 Analysis of NIH Program Activities SERIAL NO. NMI-6c
December 1955

Budget Data Sheet

16.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

None

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

None

R. P. C. - 1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

1. National Microbiological Institute
INSTITUTE

2. Laboratory of Clinical Investigation
LABORATORY OR BRANCH

3. Biochemistry
SECTION

4. Bethesda, Maryland
LOCATION

5. NMI- 7C
SERIAL NO.

6. Fundamental studies on host-parasite relationships at the cellular level.
PROJECT TITLE

7. Dr. Harry G. Steinman
PRINCIPAL INVESTIGATOR

8. Dr. Eskin Huff
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives:

The general program deals with the biochemical effects of infectious disease agents on their hosts. To study the host-parasite relationships at the cellular level, the nature of viral growth will be examined in a pure cell-line tissue culture system maintained in a chemically defined culture fluid. These studies can be divided into five parts:

Part 1/ Nutritional requirements of pure cell-lines in tissue culture are being determined so that chemically defined media may be developed for use in subsequent studies.

Part 2/ Metabolic studies of pure cell-lines in tissue culture. Certain enzymatic studies are being performed on cells grown in undefined, complex media. Other studies require the use of chemically defined media. Radio-isotope labelled metabolites will be used in these studies. Particular emphasis is being placed on the mechanism of protein biosynthesis and nucleic acid biosynthesis.

Part 3/ Nature of viral multiplication. Techniques are being evolved for the isolation and purification of viruses grown in tissue culture, preparatory to undertaking kinetic studies of viral growth in individual cells. As a model system, the APC virus is being isolated from tissue cultures of the HeLa cell and of monkey kidney cells.

Objectives: (continued)

Part 4/ Nature of viral infection. The effects of the presence of a parasitic virus on the metabolism of a host cell are being investigated using pure cell lines in tissue culture in a chemically defined medium.

Part 5/ Detection of virus. As a corollary of these studies, methods are being developed by chemical means, for the detection of viral disease agents in any biologic material. The virus of infectious hepatitis is being investigated currently.

Methods Employed:

Large stocks of several pure cell-lines are maintained on semi-synthetic media in tissue culture. Subcultures of these in their logarithmic growth phase are used in the various studies, i.e., nutritional requirements, enzymatic constitution, and viral infection.

The growth-promoting factors supplied by serum protein are isolated by fractionation of the protein. This is done by salt precipitation and alcohol precipitation. These fractions are further purified by electrophoresis and chromotography.

Encymatic studies are done on cells which are "solubilized" by sonic disintegration.

Viral studies are done on cells growing in tissue culture and infected with various purified viruses.

Patient Material:

None

Major Findings:

It has been found that a wide variety of "pure" cell-lines grown in tissue culture - both human and nonhuman and normal and malignant - have identical nutritional requirements for amino acids, vitamins, salts, etc. Above and beyond these factors, whole serum is also essential for growth. It has been established that the essential factors provided by serum are contained in the protein fraction of serum. Serum protein has been fractionated by chemical and physical means and it has been found that the growth-promoting activity is associated with one particular protein fraction. The nature of the growth-promoting activity of this protein fraction is under investigation, i.e., whether the whole protein is essential or whether the protein is a carrier of small molecular weight compounds. If the latter, these will be isolated and chemically identified.

Project Description Sheet

Significance to Microbiological Research:

One immediate practical application of having available tissue culture media for human cells containing a purified protein fraction in place of whole serum would be in the screening of patient material for the presence of viruses. Whole serum contains not only unidentified substances toxic to cell growth but also antibodies inhibitory to viral reproductions. Preliminary evidence shows that these toxic and inhibitory factors are readily separated away from the growth-promoting factors in serum protein.

Of more fundamental significance is the fact that identification of the growth factors supplied by serum protein would permit the formulation of completely defined media for tissue culture. Differences in nutritional requirements between various cell-lines would probably emerge which are not now apparent. Species-specificity, that is, the specific requirement of a particular cell for serum protein from the host animal, might well disappear. It is also possible that the unidentified growth factors may be new substances with vitamin-like activity.

Proposed Course of Project:

As described.

R. P. C. - 2 Analysis of NIH Program Activities SERIAL NO. NMI- 7C
December 1955

Budget Data Sheet

10.

11. BUDGET DATA:

ESTIMATED	BUDGETED POSITIONS			MAN YEARS			PATIENT
EXPENDITURES	PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	DAYS

12. BUDGET ACTIVITY:

Research

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 OR 1957:

None

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

None

Honors, Awards, and Publications Sheet

15.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

None

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

None

R. P. C. - 1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

1. National Microbiological Institute
INSTITUTE

2. Laboratory of Clinical Investigation
LABORATORY OR BRANCH

3. None
SECTION

4. Bethesda, Maryland
LOCATION

5. NMI- 8C
SERIAL NO.

6. Allergy-Immunology
PROJECT TITLE

7. Dr. Norman B. McCullough
PRINCIPAL INVESTIGATOR

8. Drs. Harry G. Steinman, John P. Utz, Christopher M. Martin, and Robert H. Parrott.
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives:

1. To evaluate the role of immune serum globulins in host response to infectious diseases.

2. To evaluate the role of bacterial, fungal, or viral allergens in recurrent or chronic respiratory disease.

3. To determine the rate of metabolism of individual antibodies compared to that of gamma globulin, and to investigate the mechanism whereby the reticulo-endothelial system differentiates between otherwise chemically homogeneous specific antibodies.

4. To measure, quantitatively, the rate of synthesis of synthesis of specific antibody in the human during the primary and secondary immune responses.

5. To measure quantitative differences in the specific antibody response of acute leukemics, normal human volunteers, hypo-and hypergammaglobulinemias.

Methods Employed:

Clinical studies supplemented with:

1. Plasma protein analysis by (a) electrophoresis by the paper chromatographic technique, (b) chemical methods, and (c) checking with immunologic techniques.

2. Immunization and testing of response by serologic techniques or challenge.

Methods Employed: (continued)

3. Immunochemical procedures based on the Kabat and Meyer method of analysis of specific precipitates.

Patient Material:

		No.	Average Stay Days
Admissions:	Adult males:	0	0
	Adult females	8	42
	Children males	4	42
	Children females	1	11
Outpatients:	Number of patients:	33	
	Number of visits:	396	

Major Findings:

A small number of uniquely reactive individuals have been studied:

1. One child and 3 adults with marked hypogammaglobulinemia ("Agammaglobulinemia").

2. A child with recurrent periodic hyperglobulinemia.

3. A child, with adrenal disease necessitating cortisone use, who has hypogammaglobulinemia.

It was demonstrated that a patient with hypogammaglobulinemia could be endowed with a system of active immunity by transplanting to her eight lymph nodes from a normal donor. The lymph nodes survived approximately 160 days and their antibody production was quantitated. Detailed immunochemical analyses of the response of the nodes indicated that gram for gram, human lymph nodes can produce, during the secondary immune response, specific antibody (gamma-and beta-2-globulin) at a rate of synthesis approximating that of human and animal endocrine and exocrine tissues -- 15-25 mg. protein per gram of tissue per 24 hours. This study represented the first such direct in vivo measurement of protein synthesis in man.

Evidence for an intracellular function of gamma globulin, in contrast to its already well-known extracellular function, was obtained through quantitative chemical and bacteriological studies of the sputum in a hypogammaglobulinemic adult with severe, irreversible bronchiectasis. Gamma globulin appeared to function as a regulator of bronchial protein

Project Description Sheet

Major Findings: (continued)

(mucous) and DNA (purulence) production through mechanisms independent of the severity of the extra-cellular bacterial stresses which, it had been believed, ordinarily determined the degree of host response to infection.

Data derived from detailed studies in four hypogammaglobulinemic patients indicates that specific passively-infused antibodies are metabolized at very different rates in the same individual at the same time, but that each individual metabolizes a given antibody at the same rate. Studies thus far indicate that for three different antibodies, the rate of metabolism is as follows:

	Half-Life
1. Poliomyelitis Type I neutralizing antibody	45 ± 5 days
2. Typhoid H agglutinin	35 ± 2 days
3. Coxsackie A neutralizing antibody	21 ± 2 days

Similar studies are also being conducted on APC Type II Neutralizing antibody and diphtheria and tetanus antitoxin.

Preliminary studies indicate that the differences in half-life are not artifactual (related to methods of titration) but are closely related to the electrical charge and electrophoretic mobility of the individual antibodies.

Studies in Leukemia: On a standard schedule of antigen injection and serologic testing, ten acute leukemia patients and ten normal human volunteers have been tested for differences in antibody production.

Significance to Microbiological Research:

This project exemplifies the use of clinical material in basic immunologic research. Knowledge resulting from such studies may be expected to increase our understanding of the infectious and allergenic diseases and ultimately lead to improved clinical management of patients with such diseases.

Proposed Course of Project:

Continue as outlined.

R. F. C. - 2 Analysis of NIH Program Activities SERIAL NO. NMI-8C
December 1955

Budget Data Sheet

10.

11. BUDGET DATA:

ESTIMATED	BUDGETED POSITIONS			MAN YEARS			PATIENT
EXPENDITURES	PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	DAYS

12. BUDGET ACTIVITY:

Research

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 OR 1957:

1. Division of Biologic Standards
2. Laboratory of Metabolism, National Heart Institute
3. Laboratories of Pathologic Anatomy, Biology, and Biochemistry, National Cancer Institute.
4. General Medicine Branch, National Cancer Institute.

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS) IDENTIFY SUCH RESEARCH:

This project supplements and is correlated with research done in the Laboratory of Infectious Diseases, National Microbiological Institute.

Honors, Awards, and Publications Sheet

15.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROBJECT DURING CALENDAR YEAR 1955:

(1) Martin, C. M., Gordon, R. S., and McCullough, N. B. : Acquired hypogammaglobulinemia in an adult. Report of a case, with clinical and experimental studies. New Eng. J. Med., (in press)

Papers: Martin, C. M. : Hypogammaglobulinemia in an adult. USPHS Clinical Society Regional Meeting, Staten Island, New York, June 4, 1955.

Martin, C. M. : Degradation and synthesis of gamma globulin in the human. Quantitative studies in an adult hypogammaglobulinemic. NIH Clinical Research Meeting, Bethesda, Md., October 1, 1955.

Martin, C. M. : Gamma globulin metabolism and synthesis. Conference given at Thorndike Memorial Laboratories, Boston City Hospital, Boston, Mass., October 5, 1955.

Martin, C. M. : Agammaglobulinemia. Conference given before the NIH Department of Clinical Pathology, August 11, 1955.

Martin, C. M. : Studies in agammaglobulinemia. Conference given before National Heart Institute Clinical Investigation Group, August 9, 1955.

Martin, C. M. : Degradation and synthesis of gamma globulin in the human. NIAMD Research Conference. Paper discussed by Christian B. Anfinson, NHI, November 1, 1955.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

None

R. P. C. - 1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

1. National Microbiological Institute
INSTITUTE

2. Laboratory of Clinical Investigation
LABORATORY OR BRANCH

3. None
SECTION

4. Bethesda, Maryland
LOCATION

5. NMI- 9C
SERIAL NO.

6. The Microbiologic Flora: Antibiotics (Cystic fibrosis of Pancreas)
PROJECT TITLE

7. Dr. Robert H. Parrott
PRINCIPAL INVESTIGATOR

8. None
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives:

In selected patients to study the bacterial and fungal flora particularly of the respiratory and intestinal tracts in relation to, (a) modification of flora by antibiotics and chemotherapeutic agents, (b) sensitivity of organisms to various antibiotics, and (c) prevention of the overgrowth of undesirable organisms and resultant side-reactions.

Methods Employed:

Patients with cystic fibrosis of the pancreas will be employed in this study. Long-term studies of the bacterial flora of the respiratory and intestinal tracts will be employed. Findings will be correlated with use of antibiotics and fungistatic agents, with antibiotic sensitivities of pathogenic microorganisms isolated, in particular the Staphylococci; persistence of strains of Staphylococci will be determined. A variety of metabolic studies are performed in an attempt to understand the increased pathogenicity of these organisms in this group of patients.

Patient Material:

		No.	Average Stay Days
Admissions:	Adult males	0	0
	Adult females	1	12
	Children male	5	53
	Children female	1	40
Outpatient:	Number of patients:	11	
	Number of visits:	264	

Major Findings:

In six children with cystic fibrosis of the pancreas, it has been found that: a) Staphylococcus aureus is usually present in sputum; b) the same strain remains with a patient over a period of months and is not significantly influenced by antibiosis; c) sulfonamides and, apparently, antibiotics are poorly absorbed by these children.

Significance to Microbiological Research:

With increasing availability of antibiotics, there is great need for basic guidelines for antibiotic resistant strains of bacteria. Overgrowth of undesirable bacterial and fungal organisms with resultant side reactions and sometimes fatal complications become an ever-present menace, from which medicine must protect itself and the patient.

Fibrocystic disease of the pancreas (mucoviscidosis, exocrinosis), is a recessively inherited disease manifest physiologically and clinically in any or all of the following ways:

(1) Increased viscidity of secretions from the exocrine organs (pancreas, mucous glands, certain salivary glands) with consequent:

a. Insufficiency or absence of enzymes from the pancreas, resulting in poor digestion of food, passage of large bulky stools and undernutrition; and

b. Thickened bronchial secretions associated in time with frequent severe respiratory infections, and debilitating chronic lung disease.

(2) Increased excretion of sodium and chloride in the sweat and saliva, which can result in severe heat prostration.

(3) A peculiar susceptibility to infection with staphylococci.

The incidence of the disease is 1 to 1.7 cases per 1000 live births and it is estimated that one in 20 well persons carries the recessive gene. The specific cause is not known. The diagnosis can be suspected clinically in many cases but newer methods of diagnosis based on the physiological defects mentioned are uncovering many variants from the classical case. The prognosis is still poor despite the use of antibiotics in suppressing infection.

Clinical treatment is directed toward:

(1) Maintaining nutrition
(2) Prevention and suppression of infection
(3) Prevention and alleviation of chronic lung disease

Project Description Sheet

Significance to Microbiological Research: (continued)

Clinical research on the subject is also directed toward the accomplishment of these ends. Clinical and basic research is directed toward such problems as the basic cause and cellular nature of the defect and the factors that make the children peculiarly susceptible to staphylococcal infection.

Proposed Course of Project:

As under "Objectives" and "Methods."

R. P. C. - 2
December 1955

Analysis of NIH Program Activities SERIAL No. NMI-9C

Budget Data Sheet

10.

11. BUDGET DATA:

ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
	PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	

12. BUDGET ACTIVITY:

Research

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, or OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 OR 1957:

None

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

None

Honors, Awards, and Publications Sheet

15.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

None

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

None

R.P.C. - 1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

1. National Microbiological Institute
INSTITUTE

2. Laboratory of Clinical Investigation
LABORATORY OR BRANCH

3. None
SECTION

4. Bethesda, Maryland
LOCATION

5. NMI- 10C
SERIAL NO.

6. The Physico-Chemical Properties and Reactions of Viruses
PROJECT TITLE

7. Dr. Christopher M. Martin
PRINCIPAL INVESTIGATOR

8. None
OTHER INVESTIGATOR(S)

9. PROJECT DESCRIPTION

Objectives:

As stated in the Annual Report of December, 1954 (q.v.) the objectives of this project related to fundamental studies of the physico-chemical properties of viruses purified by newer chemical methods, including investigation of solubility curves and metal-virus complexes, of the nature of viral nucleic acid-protein linkages, and of the chemical basis for viral antigenicity.

With the development in May, 1955, of urgent, practical virological problems associated with formalin-killed poliomyelitis (Salk) vaccine, the investigator was asked by Dr. Roderick Murray, Assistant Chief, Division of Biologic Standards, to participate in the newly-organized DBS program of research into methods designed to improve the safety and potency of poliomyelitis vaccine.

Accordingly, planned projects for the year were postponed temporarily, and joint studies set up with Drs. Bernice Eddy and George B. Hottle of the Division of Biologic Standards, with the following objectives:

1. Development of a more sensitive test for detection of minute amounts of residual live virus in formalinized poliomyelitis vaccines.
2. Investigation of the possible use of metal-concentrated poliomyelitis virus in the production of a poliomyelitis vaccine.

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December 1955

Analysis of NIH Program Activities SERIAL No. NMI- 10C

Project Description Sheet

Methods Employed:

1. Modification of Cohn Plasma Fractionation Method 12, employing metal-protein-chelate buffer system (Martin, C. M., Chapman, S. S., and Eaton, M. D. : Concentration and purification of PR-8 influenza virus using zinc salts. Abstracts, 54th General Meeting, Society of American Bacteriologists, May 6, 1954).

2. Standard methods of titrating poliomyelitis virus in monkey kidney roller tube tissue cultures.

Patient Material:

None

Major Findings:

1. All three vaccine strains of poliomyelitis virus (Mahoney, MEF-1, Saukett), whether present in large or trace amounts, can be precipitated with zinc salts, concentrated up to 500X, and recovered rapidly and quantitatively from both large and small volumes of various diluents, including Mixture 199, Hanks Solution, Lacto-Hanks Medium, and undialyzed, otherwise toxic trivalent formalinized poliomyelitis vaccine.

2. Very few critical conditions other than low (0 - 2°C) temperature were found: virus precipitation and total recovery could be accomplished over a wide pH range (5.6 - 7.5), a wide ionic strength range (0.05 - 0.30) and a wide zinc ion concentration range (5-25mM/L.), and in the presence of the various extraneous substances commonly found in trivalent vaccine: phenol red, monkey kidney protein, penicillin, dihydrostreptomycin, formalin, sodium bisulfite, methyl paraben, propyl paraben, thimerosal, and benzalkonium chloride.

3. By means of zinc concentration, infectious poliomyelitis virus was recovered from two vaccine preparations which had previously been tested at N.I.H. by the revised methods and found negative.

4. There is evidence that trivalent vaccines deliberately contaminated with sub-detectable amounts of a stock laboratory strain of virus, although useful as experimental models, do not fully approximate the situation that obtains in vaccines containing native infectious virus, differing from the latter in two respects:

(a) Until passed once or twice, native agents isolated from vaccines were apparently markedly inhibited by the presence of large amount of inactivated virus, whereas even in trace amounts, laboratory strains were not appreciably inhibited by inactivated virus.

(b) Following 200X concentration of vaccine, the recovery of trace amounts of a laboratory strain of Mahoney, MEF-1, or Saukett virus was quantitative, and the recovery regular and predictable. The pattern of recovery of native infectious virus, on the other hand, was not only very irregular and capricious, but in some instances revealed virus only in dilutions of the concentrate more dilute than the starting material. This finding suggests that concentration is not necessarily the key to an improved safety test; rather, it suggests the critical importance of a step in the safety test process capable of breaking up virus-concealing particulates.

5. Studies - January-May, 1955:

(a) Employing zinc salts, rabbit fibroma virus was easily purified from crude tumor suspensions, with good recovery. Electron microscope studies confirmed the high degree of purity of the product and revealed virtual absence of the viral clumping seen in crude and differential centrifugation-purified virus preparations.

(b) Stools collected from patients with acute infectious hepatitis during their first two weeks of hospitalization were extracted, zinc precipitated, and concentrated 200X. Tissue culture toxicity of the concentrates was removed by passage through a cationic exchange resin on the sodium cycle. Upon inoculation into monkey kidney, HeLa, and human amnion roller tube cultures and into fetal guinea pigs, no disease resulted.

Significance to Microbiological Research:

The study represents a practical application of newer methods in chemical virology to a serious public health problem, and suggests further practical applications in the fields of:

a) Production of human vaccines
b) Laboratory and clinical isolation of viruses from body fluids
c) Clinical diagnosis of viral diseases

Proposed Course of Project:

Studies will continue as outlined under "Objectives."

R.P.C. - 2 Analysis of NIH Program Activities SERIAL NO. NMI- 10C
December 1955

Budget Data Sheet

10.

11. BUDGET DATA:

ESTIMATED	BUDGETED POSITIONS			MAN YEARS			PATIENT
EXPENDITURES	PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	DAYS

12. BUDGET ACTIVITY:

Research

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 OR 1957:

Division of Biologics Standards, National Microbiological Institute

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

(1) The Section on Virus Vaccines and Basic Studies of the Division of Biologic Standards.

(2) The Section on Hepatitis of the Division of Biologic Standards.

(3) The Laboratory of Physiology, Physical Chemistry Section, National Cancer Institute.

Honors, Awards, and Publications Sheet

15.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

None

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

Served on Poliomyelitis Advisory Sub-committee on Concentration, Joseph E. Smadel, Chairman.

R. P. C. - 1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

1. National Microbiological Institute
INSTITUTE

2. Laboratory of Clinical Investigation
LABORATORY OR BRANCH

3. None
SECTION

4. Bethesda, Maryland
LOCATION

5. NMI- 11C
SERIAL NO.

6. Blood and Tissue Protozoan Diseases
PROJECT TITLE

7. Dr. Henry K. Beye
PRINCIPAL INVESTIGATOR

8. Dr. Donald E. Kayhoe and Miss Elizabeth Guinn
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives:

To improve diagnostic techniques; to study the clinical and pathological manifestations of these diseases in man and their pathogenesis; to evaluate various experimental therapeutic and prophylactic regimens.

Methods Employed:

Field surveys of groups of people, as indicated; parasitic disease work-up of patients referred as suspected cases by private physicians or governmental organizations; clinical study of proved cases; laboratory diagnostic techniques, including cytodiagnosis; immunologic studies; laboratory culture of the organisms; and experimental therapy.

Patient Material:

None

Major Findings:

None. This is a proposal for a new project.

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December 1955

Analysis of NIH Program Activities SERIAL NO. NMI-11C

Project Description Sheet

Significance to Microbiological Research:

The study of these groups of diseases has significance in terms of their prevalence, and of the application of newer fundamental concepts and techniques to studies of host-parasite relation, immunological reactions, pathogenicity, and therapy.

The first case of Chagas disease in the United States has recently been reported in Texas. Suitable insect vectors for the transmission of the disease are distributed throughout most of the Southwestern and Western parts of the United States.

Proposed Course of Project:

As time and staffing allows, to obtain information on the epidemiological and clinical aspects of the disease, and then proceed as described.

R. P. C. - 2
December 1955

Analysis of NIH Program Activities

SERIAL NO. NMI- 11C

Budget Data Sheet

10.

11. BUDGET DATA:

ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
	PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	

12. BUDGET ACTIVITY:

Research

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 OR 1957:

None

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

This project supplements and is correlated with research done in the Laboratory of Tropical Diseases, National Microbiological Institute.

R. P. C. - 3 Analysis of NIH Program Activities SERIAL No. NMI- 11C
December 1955

Honors, Awards, and Publications Sheet

15.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

None

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

None

R.P.C. - 1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

1. National Microbiological Institute
INSTITUTE

2. Laboratory of Clinical Investigation
LABORATORY OR BRANCH

3. None

4. Bethesda, Maryland
LOCATION

5. NMI - 12C
SERIAL NO.

6. Q Fever
PROJECT TITLE

7. Dr. John P. Utz
PRINCIPAL INVESTIGATOR

8. None
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives:

To evaluate Q fever as a cause of chronic disease; to study chemotherapy on patients with presumed residua of previous illness; to study basic immunology of long term complement-fixation antibody levels.

Methods Employed:

Hospitalization of acutely ill patients who have had chronic complaints post-infection; isolation attempts using animal, chick embryo and tissue culture techniques; clinical observation with laboratory studies of chemotherapy response.

Patient Material:

		No.	Average Stay Days
Admissions:	Adults	0	0
	Children	0	0

Outpatient: Number of patients 11
Number of visits 55

R.P.C. - F
December 1955

Analysis of NIH Program Activities SERIAL NO. NMI 12C

Project Description Sheet

Major Findings:

Many patients with old Q fever have a number of symptoms, which, though not specific for Q fever, are similar and characterizable. Most such patients have continuing complement fixing antibodies despite no known new exposure to the organism. The degree of chronic damage can be assessed and patients helped back to working daily in some cases by use of antibiotics or other therapy.

Significance to Microbiological Research:

All patients studied thus far in this project are NIH employees, some of whom are on disability retirement. The results of these studies should have application to the general field of rickettsial illness, some of which are important causes for military service disability.

Proposed Course of Project:

Continuation as a project on the same level as previously.

R. P. C. - 2 Analysis of NIH Program Activities SERIAL NO. NMI- 12C
December 1955

Budget Data Sheet

10.

11. BUDGET DATA:

ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
	PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	

12. BUDGET ACTIVITY:

Research

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 OR 1957:

None

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

This project supplements and is correlated with research done in the Laboratory of Infectious Diseases, National Microbiological Institute.

R.P.C. - 3 Analysis of NIH Program Activities SERIAL NO. NMI 12C
December 1955

Honors, Awards, and Publications Sheet

15.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

None

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955.

None

R.F.C. - 1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

1. National Microbiological Institute
INSTITUTE

2. Laboratory of Clinical Investigation
LABORATORY OR BRANCH

3. None
SECTION

4. Bethesda, Maryland
LOCATION

5. NMI- 13C
SERIAL NO.

6. Non-Bacterial Meningo-encephalitis
PROJECT TITLE

7. Dr. John P. Utz
PRINCIPAL INVESTIGATOR

8. Dr. Robert H. Parrott, and Dr. Lewis E. Gibson
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives:

a) To develop methods and use newly available methods to diagnose the large group of non-bacterial meningo-encephalitides.

b) To correlate etiological findings with detailed historical, physical and clinical laboratory observations.

c) To study poliomyelitis in pregnancy in relation to the infant.

d) To further define existing etiological entities and to discover new etiological entities in this group of diseases.

Methods Employed:

Hospitalization of patients for clinical observation and laboratory studies. Laboratory procedures include: a) tissue culture and suckling mouse inoculation of material for virus isolation; b) appropriate serological procedures with acute and convalescent serum for the known viral meningo-encephalitides, leptospira, toxoplasma and infectious mononucleosis.

Patient Material:		No.	Average Stay Days
Admissions:	Adult males	10	19
	Adult females	7	30
	Children male	3	36
	Children female	2	23
Outpatient:	Number of patients:	22	
	Number of visits	66	

Project Description Sheet

Major Findings:

Group B Coxsackie infection has been found responsible for aseptic meningitis in a family outbreak and in several isolated cases. The clinical attributes of this virus infection have been further delineated. Poliomyelitis virus was not isolated from the fetus of an abortion during active poliomyelitis.

Significance to Microbiological Research:

Non-bacterial meningitis is a serious disease of whose etiology and pathogenesis there is little knowledge. It often results in brain damage and behavior disturbances. Increased knowledge may also clarify the pathogenesis of the encephalitic complications of the otherwise minor contagious diseases of children.

Proposed Course of Project:

Continue above studies with greater effort to study material at other hospitals in the Washington area.

R. P. C. - 2 Analysis of NIH Program Activities SERIAL NO. NMI-13C
December 1955

Budget Data Sheet

10.

11. BUDGET DATA:

ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
	PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	

12. BUDGET ACTIVITY:

Research

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 OR 1957:

Research Foundation of Children's Hospital, Washington, D. C.

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

This project supplements and is correlated with research done in the Laboratory of Infectious Diseases, National Microbiological Institute.

Honors, Awards, and Publications Sheet

15.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

(1) Burke, Frederic G., Ross, Sydney, Parrott, Robert H., and Rice, E. Clarence. : Nontuberculous Bacterial Meningitis in Infants and Children. General Practitioner, XII(5): 98-103, November 1955.

(2) Utz, John P. : A Clinical Study of Poliomyelitis in The Tropics. Annals of Int. Med., 42(6): 1190-1197, June, 1955.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

None

R. P. C. - 1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

1. National Microbiological Institute
INSTITUTE

2. Laboratory of Clinical Investigation
LABORATORY OR BRANCH

3. None
SECTION

4. Bethesda, Maryland
LOCATION

5. NMI-14C
SERIAL NO.

6. Respiratory Viral Diseases
PROJECT TITLE

7. Dr. Robert H. Parrott
PRINCIPAL INVESTIGATOR

8. Dr. John P. Utz
OTHER INVESTIGATOR

9. PROJECT DESCRIPTION

Objectives:

(a) To define clinical entities in relation to newly isolated respiratory viruses.

(b) Further to define diagnostic criteria, pathogenesis, immune response, persistence and sites of virus and effects on tissues of virus in certain infections of respiratory passages and mouth.

(c) To study host-parasite relationships in reference to susceptibility to chronic or recurrent respiratory disease.

Methods Employed:

Results of clinical observations and procedures are correlated with bacterial, mycologic and viral isolation employing both animals and tissue culture techniques.

Patient Material:		No.	Average Stay Days
Admissions:	Adult males	14	19
	Adult females	11	11
	Children males	32	24
	Children females	12	12
Outpatient:	Number of patients:	189	
	Number of visits:	567	

Major Findings:

1. The Laboratory of Clinical Investigation diagnostic viral Laboratory has reached maturity in that standard animal and tissue culture methods and many of the stock viruses and serological reagents, have been made available.

2. No known or unknown virus agent was found in over 100 NIH employees with acute minor respiratory disease. This includes, APC, herpes, psittacosis, and influenza agents.

3. Tonsils and Adenoids. In 19 children whose tonsils and/or adenoids were removed, an APC virus was recovered from these tissues in 15 instances and a varicella-like virus in one instance. The Table summarizes attempts to correlate presence of virus with certain clinical features.

	VIRUS ISOLATED			Sinus Involvement	Narrow Naso-Pharynx
	Tonsil	Adenoid	N-P Swab		
Tonsillectomy & Adenoidectomy (10)	8	6	0	4	7
Tonsillectomy (4)	4	x	0	0	0
Adenoidectomy (5)	x	2	0	2	4

4. Chronic Recurrent Stomatitis. In 12 children and adults, evidence has been accumulated that their disorder is not regularly associated with direct or indirect evidence of infection. The impression is gained that emotional factors strongly influence the occurrence of ulcers.

Project Description Sheet

Significance to Microbiological Research:

Illustrates progress in study of respiratory disease, acute and chronic, and oro-pharyngeal ulcerative disease which are prime unsolved problems in the infectious disease field. The major advances in this field are being made in settings such as this where the efforts of research laboratories in bacteriology, mycology and virology can be correlated with clinical material under ideal study conditions.

A major effort is exerted toward clinical studies in relation to newer viruses uncovered by tissue culture techniques.

Proposed Course of Projects:

Studies will continue as outlined under "Objectives" in patients referred to the Clinical Center, those seen in the Respiratory Disease Clinic (Employee's Health Unit) and in other hospital or community settings when indicated.

R. P. C. - 2
December 1955

Analysis of NIH Program Activities SERIAL NO. NMI-14C

Budget Data Sheet

10.

11. BUDGET DATA:

ESTIMATED	BUDGETED POSITIONS			MAN YEARS			PATIENT
EXPENDITURES	PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	DAYS

12. BUDGET ACTIVITY:

Research

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, or OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 OR 1957:

Research Foundation of Children's Hospital, Washington, D. C.

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

This project supplements and is correlated with research done in the Laboratory of Infectious Diseases, National Microbiological Institute.

Honors, Awards, and Publications Sheet

15.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

(1) Bell, Joseph A., Rowe, Wallace P., Engler, Joseph I., Parrott, Robert H., and Huebner, Robert J. : Pharyngoconjunctival Fever. Epidemiological Studies of a Recently Recognized Disease Entity. J.A.M.A., 157: 1083-1092, March 26, 1955.

(2) Parrott, Robert H., Huebner, Robert J., McCullough, Norman B., Wolf, Stanley I., and Naiden Eulaine, The Hospital as a Factor in Assessing the Occurrence of Coxsackie Viruses in Various Illness Groups: I. Children From the Outpatient Department and Various Hospital Wards. Pediatrics, 15(3): 255-263, March, 1955.

(3) Rowe, Wallace P., Huebner, Robert J., Hartley, Janet W., Ward, Thomas G., and Parrott, Robert H. : Studies of the Adenoidal-Pharyngeal-Conjunctival (APC) Group of Viruses. Amer. J. Hyg., 61(2): 197-218, March, 1955.

(4) Parrott, Robert H. : Herpangina and Herpetic Gingivostomatitis; Clinical Differentiation. Postgraduate Medicine, 17(4): 298-301, April, 1955.

(5) Parrott, Robert H. : Adenoidal-Pharyngeal-Conjunctival Viruses in Common Respiratory Diseases. Clinical Proceedings of the Children's Hospital, Washington, D. C., XI(7): 152-158, July 1955.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955.

None

R.F.C. - 1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

1. National Microbiological Institute
INSTITUTE

2. Laboratory of Clinical Investigation
LABORATORY OR BRANCH

3. None
SECTION

4. Bethesda, Maryland
LOCATION

5. NMI- 15C
SERIAL NO.

6. Enteric Diseases
PROJECT TITLE

7. Dr. Norman B. McCullough
PRINCIPAL INVESTIGATOR

8. Dr. Robert H. Parrott, Dr. Thomas L. Gorsuch, and Miss Rose Lieberman
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives:

1. Determination of the etiology of undiagnosed enteric infections in children and adults.

2. Study of the pathogenesis and pathophysiology of the asymptomatic carrier state in Salmonella and Shigella infections and the effects of these chronic infections on the economy of the hosts.

3. Study of antibiotic, surgical, and immunologic therapy of Salmonella and Shigella carrier states.

Methods Employed:

Standard bacteriologic and viral techniques and newer tissue culture and immunologic methods will be employed in etiologic studies and investigation of the carrier state.

Patient Material:

		No.	Average Stay Days
Admissions:	Adult males	7	49
	Adult females	6	51
	Children males	6	22
	Children females	8	52
Outpatient:	Number of patients:	59	
	Number of visits:	177	

Project Description Sheet

Major Findings:

Etiologic studies were made on a number of patients with acute and chronic enteritis. Four patients with Salmonella enteritis were successfully treated with antibiotics. One typhoid carrier was completely cured by a new combination of antibiotics. A large outbreak of Salmonella infections was studied and the preliminary data necessary for an adequate study of the carrier state were obtained. Present concepts suggest that immunologic defects, possibly remediable, may be causally related to the development of the carrier state.

Significance to Microbiological Research:

Outbreaks of enteritis, often of unknown etiology, are of major importance, particularly when children are involved. Carriers who have chronic asymptomatic infections are usually responsible for these outbreaks, and are a serious public health problem. The carrier state does not yield to present methods of therapy.

Proposed Course of Project:

Etiologic studies on patients from epidemics and sporadic cases of enteritis will continue.

Major emphasis will be placed on an investigation of the etiology and treatment of the carrier state in Salmonella and Shigella infections. In particular, the possible roles of allergy and immunologic abnormalities in the pathogenesis of these infections will be studied. Extensive therapeutic investigations are planned utilizing antibiotics, singly and in combination, and measures which will alter the immunologic state of the host.

R.P.C. - 2 Analysis of NIH Program Activities SERIAL NO. NMI-15C
December 1955

Budget Data Sheet

10.

11. BUDGET DATA:

ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
	PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	

12. BUDGET ACTIVITY:

Research

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATION PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 OR 1957:

None

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH.

None

Honors, Awards, and Publications Sheet

15.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

None

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

None

R. P. C. - 1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

1. National Microbiological Institute
INSTITUTE

2. Laboratory of Clinical Investigation
LABORATORY OR BRANCH

3. None
SECTION

4. Bethesda, Maryland
LOCATION

5. NMI- 16C
SERIAL NO.

6. Amebiasis
PROJECT TITLE

7. Dr. Henry K. Beye
PRINCIPAL INVESTIGATOR

8. Dr. Donald E. Kayhoe and Miss Elizabeth Guinn
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives:

To improve and standardize diagnostic techniques for amebiasis due to Endamoeba histolytica; to determine adequate criteria for the diagnosis of amebiasis; to investigate differences in strains or races of the parasite in correlation with clinical attributes; to study the role of associated bacterial flora; to study the clinical and pathological manifestations of the disease in man and its pathogenesis; to evaluate various experimental therapeutic regimens and to establish valid criteria of cure.

Methods Employed:

Field surveys of groups of people for intestinal parasites, parasitic disease work-up of patients referred by private physicians or governmental organizations as suspected cases of amebiasis, clinical study of proved cases, laboratory diagnostic techniques, immunological studies, laboratory culture of the parasite and associated microorganisms, experimental therapy.

Patient Material:

		No.	Average Stay Days
Admissions:	Adult males	6	10
	Adult females	3	10
	Children males	1	68
	Children females	0	0
Outpatient:	Number of patients	53	
	Number of visits	318	

Project Description Sheet

Major Findings:

The percentage of persons positive for E. histolytica was approximately the same in the patients referred as suspected cases of amebiasis (patients with extensive travel histories, gastrointestinal symptoms and signs, often histories of previously diagnosed amebiasis of long duration, and usually a long and varied series of treatments), as in the groups surveyed for intestinal parasites. The percentage of persons positive for E. histolytica are as follows: Patients referred by physicians: 13%, NIH employees with trichomonas: 8%, Foreign students, Howard University: 10%. Clinical trials using tetracycline, oxytetracycline, and erythromycin with chloroquine used adjunctively were continued. The series is yet too small to justify definite conclusions.

Significance to Microbiological Research:

Amebiasis continues to be a significantly large problem in the United States. Approximately ten percent of the population is estimated to harbor this organism. Factors in the pathogenesis of the disease in man, which can produce manifestations ranging from no apparent disease to an acute fulminating condition, are relatively unknown. Diagnostic techniques and criteria remain unsatisfactory; therapeusis remains largely experimental.

Proposed Course of Project:

Further development of parasitic surveys to build up a reservoir of suitable patient material. Insofar as time and staffing allows, the following aspects will be emphasized in the current year: (a) Development of improved laboratory diagnosis and exploration of feasibility of polyethylene or other plastic material in stool concentration techniques; (b) intensive study of selected patients with E. histolytica to determine the respective roles of nutrition and intestinal flora in pathology and symptomatology.

R. P. C. - 2 Analysis of NIH Program Activities SERIAL NO. NMI-16C
December 1955

Budget Data Sheet

10.

11. BUDGET DATA:

ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
	PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	

12. BUDGET ACTIVITY:

Research

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 OR 1957:

Student Health Service, Howard University, Washington, D. C.

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

This project supplements and is correlated with research done in the Laboratory of Tropical Diseases, National Microbiological Institute.

Honors, Awards, and Publications Sheet

15.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

(1) McCullough, Norman B. : The Research Approach to Clinical Problems in Amoebiasis. Amer. Jour. Gastroenterology, 23(4): 299-302, April, 1955.

(2) Imboden, Clarence A. and Beye, Henry K. : Clinical Aspects of Amebiasis with Observations on Variations in Tropical and Temperate Zones. American Jour. Gastroenterology, 23(4): 311-324, April, 1955.

(3) Imboden, Clarence A. : Chapter entitled, "Clinical Notes: On the Etiology, Pathology, Clinical Management and Therapy of Amoebiasis" in the Textbook "Problems in Amoebiasis," by Charles W. Rees, published by Chas. C. Thomas Publishers, 1955.

(4) Exhibit entitled, "Pulmonary Manifestations of Parasitic Diseases," presented at the National Tuberculosis Association, Milwaukee, Wisconsin, May, 1955; and the District of Columbia Medical Society, Washington, D. C., October, 1955.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

None

R. P. C. - 1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

1. National Microbiological Institute
INSTITUTE

2. Laboratory of Clinical Investigation
LABORATORY OR BRANCH

3. None
SECTION

4. Bethesda, Maryland
LOCATION

5. NMI- 17C
SERIAL NO.

6. Sarcoidosis
PROJECT TITLE

7. Dr. Norman B. McCullough
PRINCIPAL INVESTIGATOR

8. Dr. Horace W. Bernton and Dr. Richard Merchant
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives:

(1) Combined clinical-laboratory project to evaluate the role of immunologic mechanisms in the pathogenesis of sarcoidosis.

(2) Attempt to further define sarcoidosis as a clinical entity

(3) Study of metabolic and pathologic changes which occur in sarcoid, e.g., elevated serum calcium, relative anergy to delayed type skin test antigens, etc.

(4) Evaluation of cortisone as a therapeutic measure in sarcoidosis.

(5) Evaluation of the Kviem skin test as a diagnostic and prognostic tool in the management of sarcoidosis.

(6) Attempts to isolate pathogenic agents from patients with sarcoid.

Methods Employed:

(1) Hospitalization and outpatient observation of patients with sarcoidosis.

(2) Tissue culture of lymph nodes removed from such patients in an effort to:

(a) Study the characteristic aspects of growth of such tissue.
(b) To study cytotoxic effects of tuberculin on such tissue.

(3) Passage of tissue culture fluids from Method 2, above, to animals and other tissue culture systems in an effort to isolate pathogens.

R. F. C. - 1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

SERIAL NO. NMI-17C

Patient Material:

		No.	Average Stay Days
Admissions:	Adult males	14	25
	Adult females	14	43
	Children males	1	30
	Children females	0	0
Outpatients:	Number of patients:	46	
	Number of visits:	552	

Major Findings:

Approximately 20 cases of sarcoidosis meeting the most strict diagnostic criteria have been accumulated and thoroughly studied clinically. The patients will be continually available. Lymph node tissue explants from many of these patients have been studied growing in the presence and absence of various antigens. Marked differences have been noted in comparison with controls. Thus case material and a method for investigation of basic cellular immunology in this disease has been developed.

Significance to Microbiological Research:

Sarcoidosis is a disease of unknown cause. The pathologic picture strongly suggests that it may be of infectious origin. However, the clinical aspects of the disease and the pathologic alteration of tissues is not inconsistent with an altered response to one or more antigenic materials. This baffling disease offers an unique opportunity to study basic problems in allergy and immunology in the group of granulomatous diseases.

Proposed Course of Project:

In addition to the points covered under objectives and methods, major emphasis will be directed toward use of the tissue culture method mentioned above in explorations of phenomena associated with these noted differences in cellular immunity.

R. P. C. - 2 Analysis of NIH Program Activities SERIAL NO. NMI-17C
December 1955

Budget Data Sheet

10.

11. BUDGET DATA:

ESTIMATED EXPENDITURES	PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	PATIENT DAYS

12. BUDGET ACTIVITY:

Research

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 OR 1957:

None

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

None

Honors, Awards, and Publications Sheet

15.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

None

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

None

R. P. C. - 1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

1. National Microbiological Institute
INSTITUTE

2. Laboratory of Clinical Investigation
LABORATORY OR BRANCH

3. None
SECTION

4. Bethesda, Maryland
LOCATION

5. NMI- 18C
SERIAL NO.

6. Systemic Fungus Disease: (a) Histoplasmosis
(b) Moniliasis
(c) Cryptococcosis
(d) Related fungus infections
PROJECT TITLE

7. Dr. Norman B. McCullough
PRINCIPAL INVESTIGATOR

8. Dr. Donald B. Louria, Dr. John P. Utz, Dr. Robert H. Parrott,
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives:

A. To study clinical diagnosis, pathogenesis and natural course of systemic fungus infections.

B. To study the problem of fungus disease due to overgrowth during antibiotic treatment.

C. To evaluate new agents in antibiotic and chemotherapeutic treatment of systemic fungus infections.

D. To set up means of measuring blood levels of new antifungal agents and to study acute and long term toxic effects of the drugs.

E. To study various immunologic aspects of this group of diseases.

Methods Employed:

Patients with suspected fungal infections are studied diagnostically using the skin test, serologic, histologic, and cultural methods. Family contacts are similarly investigated and environmental studies undertaken, when indicated. Infecting fungi from such patients are screened for sensitivity against a considerable number of chemotherapeutic or antibiotic agents and therapy is undertaken after such studies and where clinically advisable.

Drugs are screened for activity against an organism isolated from a patient by in vitro methods. Promising drugs are then tested in animals for acute and long term toxicity and for therapeutic effectiveness.

Methods Employed: (continued)

New methods are being developed for determining serum drug levels, rates of excretion of drug, and *in vitro* screening of drugs.

Patient Material:

		No.	AVERAGE STAY DAYS
Admissions:	Adult males	7	66
	Adult females	10	34
	Children males	3	23
	Children females	6	26
Outpatient:	Number of Patients:	62	
	Number of Visits:	744	

Major Findings:

A total of 28 patients have been studied. Thirteen of these with culturally proved systemic infections were investigated intensively; being patients with histoplasmosis, cryptococcosis, nocardiosis, aspergillosis, and coccidioidomycosis. Experimental therapy was employed. Few satisfactory therapeutic responses have been encountered. In histoplasmosis one new drug currently advocated was employed in dosages tremendously higher (18 times) than recommended; was non-toxic but also non-effective. Several other drugs are under current study.

In laboratory studies improved methods have been developed for measuring levels of several antibiotics and drugs in serum and other body fluids. Many sera appear to have non-specific inhibitory properties against a number of fungi.

Significance to Microbiological Research:

The group of fungus diseases under study is poorly understood; there is no uniformly successful treatment. Apparently, certain of them, such as histoplasmosis, are being recognized as quite prevalent; others such as moniliasis create problems by the very ubiquity of the organism, which may grow more abundantly and cause systemic disease in the presence of most antibiotics and steroids creating serious and extensive clinical problems.

Proposed Course of Project:

To proceed as outlined under "Objectives" and "Methods."

R. P. C. - 2 Analysis of NIH Program Activities SERIAL NO. NMI-18C
December 1955

Budget Data Sheet

10.

11. BUDGET DATA:

ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
	PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	

12. BUDGET ACTIVITY:

None

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 OR 1957:

None

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS) IDENTIFY SUCH RESEARCH:

This project supplements and is correlated with research done in the Laboratory of Infectious Diseases, National Microbiological Institute.

R. P. C. - 3 Analysis of NIH Program Activities SERIAL NO. NMI- 18C
December 1955

Honors, Awards, and Publications Sheet

16.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

None

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

None

R. P. C. - 1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

1. National Microbiological Institute
INSTITUTE

2. Laboratory of Clinical Investigation
LABORATORY OR BRANCH

3. None
SECTION

4. Bethesda, Maryland
LOCATION

5. NMI- 19C
SERIAL NO.

6. Infectious Hepatitis: Virus hepatitis - Serum hepatitis
PROJECT TITLE

7. Dr. John P. Utz
PRINCIPAL INVESTIGATOR

8. Dr. Robert H. Parrott and Dr. Christopher M. Martin
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives:

(1) Better to define the diagnosis, differential diagnosis and natural course of infectious hepatitis and homologous serum hepatitis.

(2) To attempt to isolate and propagate the causative agents in tissue culture.

(3) To investigate factors which predispose to chronic hepatitis or post hepatitic cirrhosis.

(4) To study antibody production during acute and convalescent stages of disease.

Methods Employed:

Clinical observation and clinical laboratory data are compiled on Clinical Center patients. Family contacts are examined and specimens obtained for clinical laboratory procedures in an attempt to define subicteric infection. Similar liver function tests are obtained on most of the febrile patients admitted to the Service for control and for evaluation of hepatitis in other entities. Tissue culture and virus purification techniques are employed in an attempt to isolate viral agents. Appropriate procedures are employed to assess the role of other organisms such as leptospira and bacteria in hepatitis.

Analysis of NIH Program Activities SERIAL NO. NMI-19C

Project Description Sheet

Patient Material:

		No.	Average Stay Days
Admissions:	Adult males	8	37
	Adult females	3	13
	Children males	2	19
	Children females	4	22
Outpatient:	Number of patients:	37	
	Number of visits:	74	

Major Findings:

There have been too few patients yet for evaluation of data. Findings on electrophoretic analysis are reported under another project, utilizing these same patients.

Significance to Microbiological Research:

The clinical spectrum of infectious hepatitis is not well defined by objective methods, but apparent infection is responsible for serious illness and death in all age groups. The frequency and clinical nature of subicteric infection is not known, but on the basis of high gamma globulin protective ability, a majority of adults have been exposed to the causative agent.

Proposed Course of Project:

To continue as outlined under "Objectives" and "Methods" on larger numbers of patients.

R. P. C. - 2 Analysis of NIH Program Activities SERIAL NO. NMI- 19C
December 1955

Budget Data Sheet

10.

11. BUDGET DATA:

ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
	PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	

12. BUDGET ACTIVITY:

Research

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 OR 1957:

Division of Biologic Standards, National Microbiological Institute.

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

None

R. P. C. - 3 Analysis of NIH Program Activities SERIAL NO. NMI- 19C
December 1955

Honors, Awards, and Publications Sheet

15.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

None

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

None

R. P. C. - 1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

1. National Microbiological Institute
INSTITUTE

2. Laboratory of Clinical Investigation
LABORATORY OR BRANCH

3. None
SECTION

4. Bethesda, Maryland
LOCATION

5. NMI- 20C
SERIAL NO.

6. Trichomoniasis
PROJECT TITLE

7. Dr. Henry K. Beye
PRINCIPAL INVESTIGATOR

8. Dr. Donald E. Kayhoe and Miss Elizabeth Guinn
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION:

This project represents a part of the total trichomoniasis program, and covers that section of the program in which there is joint participation between the Laboratory of Tropical Diseases, and the Laboratory of Clinical Investigation.

The total trichomoniasis program consists of five parts:

1. Prevalence survey
2. Preliminary studies of 150 infected volunteers
3. Pathophysiology of the vaginal tract
4. Evaluation of treatment
5. Epidemiological studies

Investigations related to the prevalence survey will be handled through collaboration with the Laboratory of Tropical Diseases, and the National Cancer Institute.

The remaining investigations are being done jointly by the Laboratory of Tropical Diseases and the Laboratory of Clinical Investigation team.

Objectives:

To define the extent of trichomoniasis and moniliasis among a population of working women; to correlate, if possible, the association of trichomoniasis and abnormal vaginal cytopathology and genital cancer; to gain epidemiological data concerning the disease; to correlate signs and symptoms of infection and presence of *Trichomonas vaginalis* and *Monilia albicans*; to ascertain the relationship of laboratory and

Objectives: (continued)

clinical findings during various phases of the menstrual cycle to infection with trichomonas and/or Monilia; to evaluate various therapeutic regimens.

Methods Employed:

Clinical, laboratory, and epidemiological studies of approximately 150 volunteers at NIH found to be positive for Trichomonas vaginalis; detailed clinical and laboratory investigation of 50 selected women with the parasite, and 20 women without Trichomonas infection at frequent intervals during one menstrual cycle (5 weeks); therapeutic trials of various treatment regimens; and detailed epidemiologic studies of family contacts.

Patient Material:

		No.	Average Stay Days
Admissions:	Adults	0	0
	Children	0	0
Outpatient:	Number of patients:	48	
	Number of visits:	192	

Major Findings:

Trichomonas vaginalis infection is seven times as prevalent in Negro employees at NIH as white employees, the percent of positive cultures being respectively 64% and 9%. Moniliasis, on the other hand, is more prevalent among white female employees, being 35% as compared to 24%.

To date, all women with Trichomonas infection who have been observed at frequent intervals for more than one month have exhibited, on one or more examinations, signs of inflammatory changes of various degrees.

Women at NIH with vaginal moniliasis exhibited a lower incidence of T. vaginalis than those without a monilial infection.

Major Findings: (continued)

All women with Trichomonas vaginalis studied at NIH have had viable T. vaginalis on external genitalia. The organism has been cultivated from pieces of wash cloth used to cleanse the external genitalia.

Eight drugs so far tested have been unsuccessful in eliminating the parasite. With those drugs causing a transient disappearance of the parasite, the first subsequent positive culture usually was obtained from the urethra.

Significance to Microbiological Research:

Trichomoniasis is a very common and occasionally severe infection among a large percentage of women in the United States. Little is known on its epidemiology, especially factors involved in transmission, and its clinical spectrum remains unclarified. To date, there is no satisfactory treatment.

Proposed Course of Project:

Epidemiologic and clinical investigations among the women, found to be positive for Trichomonas vaginalis in the prevalence survey, have been in progress during recent months. These have been conducted on an informal cooperative basis between the Laboratory of Tropical Diseases and the Laboratory of Clinical Investigation. The effort has now grown sufficiently large to formalize it as a project.

Immediate emphasis will be placed on the second part of the total program, and then on the basis of an appraisal at the completion of the preliminary survey. The remaining parts will be investigated as described.

R. P. C. - 2 Analysis of NIH Program Activities SERIAL No. NMI- 20C
December 1955

Budget Data Sheet

10.

11. BUDGET DATA:

ESTIMATED	BUDGETED POSITIONS			MAN YEARS			PATIENT
EXPENDITURES	PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	DAYS

12. BUDGET ACTIVITY:

Research

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 OR 1957:

None

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

This project supplements and is correlated with research done in the Laboratory of Tropical Diseases, National Microbiological Diseases.

Honors, Awards, and Publications Sheet

15.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

(1) Burch, Thomas; Rees, Charles; and Reardon, Lucy: Studies on Epidemiology of Trichomoniasis. Paper presented to the American Society of Tropical Medicine and Hygiene in Boston, November, 1955.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955.

None

Analysis of NIH Program Activities

Project Description Sheet

1. National Microbiological Institute
INSTITUTE

2. Laboratory of Clinical Investigation
LABORATORY OR BRANCH

3. None
SECTION

4. Bethesda, Maryland
LOCATION

5. NMI- 21C
SERIAL NO.

6. Nematode infestations
PROJECT TITLE

7. Dr. Henry K. Beye
PRINCIPAL INVESTIGATOR

8. Dr. Donald E. Kayhoe and Miss Elizabeth Guinn
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives:

To correlate clinical manifestations and pathology during the various phases of the life cycle of nematodes in the mammalian host, especially man; to describe their role in the production of disease; to evaluate the efficacy of established or newer antihelminthics; to investigate differences in species in terms of their clinical attributes; and to improve diagnostic techniques.

Among intestinal Nematode infestations, the program embraces those species whose life cycles include a period of tissue invasion and pulmonary migration (Ascaris lumbricoides, Strongyloides stercoralis, and hookworm), as well as those species which do not exhibit a pulmonary cycle (Enterobius vermicularis and Trichuris trichura). Reactions of the "tissue larva migrans" type are also of interest.

Among blood and tissue nematode infestations are included filariasis due to various species (Wuchereria bancrofti, W. malayi, L. loa, O. volvulus) and trichinosis.

Project Description Sheet

Methods Employed:

Field surveys of selected groups of people for nematode infestations; parasitic disease workup of patients referred by private physicians, or governmental organizations, for suspected nematode infestations; routine stool examinations on inpatients under investigation by the Laboratory of Clinical Investigation, National Microbiological Institute; laboratory diagnostic techniques; immunological studies; entomological investigations; tissue culture techniques; and studies in therapy.

Patient Material:

		No.	Average Stay Days
Admissions:	Adult males	1	4
	Adult females	1	6
	Children	0	0
Outpatients:	Number of patients:	4	
	Number of visits:	12	

Major Findings:

In a pilot survey of foreign students at Howard University, five of 54 students, or approximately 10%, were found to harbor microfilariae of W. bancrofti in their venous blood, when collected at night and concentrated by the method of Knott. It was demonstrated that the common mosquito vector, Culex quinquefasciatus, is capable of transmitting the disease after feeding upon one of these students having a very low density of microfilaria. (An average of 0.7 microfilaria per 20 cmm.)

Therapeutic trials and follow-up studies were carried out on patients with ascariasis, enterobiasis, and strongyloides and examinations and follow-up observations on acute filarial lymphangitis and the carrier state. These series still remain too small to warrant definite conclusions.

Significance to Microbiological Research:

Evidence indicating there could well be large numbers of carriers of filariasis, not only in the Washington area but in the United States, has major significance in many ways. The insect vector is common in the United States, so it is conceivable that foci may have already been established, but the disease not yet become clinically manifest, especially when the index of suspicion is nil. Aside from a small focus, last reported in Charlestown, South Carolina, 15 years ago, the disease has never been reported to occur in the United States. Filariasis due to W. bancrofti and W. malayi, and its well-known sequel, elephantiasis, is extremely important in many areas of the world and was of considerable significance to American troops during World War II. A great deal remains unknown about the disease and the discovery that patient material is available in the United States presents many research opportunities.

Infestations with the intestinal nematodes, especially Ascaris, is common in the United States, almost universal in much of the world and constitutes an enormous problem. Effect of the tissue phases of the parasites is suspected of being responsible for pulmonary and liver disease.

Proposed Course of Project:

In general, to continue the present program, but insofar as time and staffing allows to develop additionally as indicated in the following specific areas:

1. Filariasis:

a. Define the extent of filariasis in the Washington area, determine if transmission is occurring, and discover if indigenous cases exist; determine if the same situation exists in one or two other suitable areas in the United States.

b. Initiate attempts to find a suitable laboratory animal for the study of this disease. The lack of such an animal has greatly hindered work in this field, and very recent information from Malaya suggests that the dog, cat, and monkey may serve as reservoirs for W. malayi.

c. Investigate the feasibility of tissue culture technique in assaying cellular hypersensitivity to the products of the adult worm.

Project Description Sheet

Proposed Course of Project: (continued)

2. Intestinal Nematodes:

a. Emphasize the study of visceral "larval migrans" and pulmonary manifestations of these nematodes. To secure adequate patient material, it will be necessary to affiliate with a group doing mass chest X-rays on young children, and do concommitant stool surveys for intestinal parasites.

b. In addition to investigating tissue culture techniques, as in item (c) above, a study of the viruses in nematodes should be undertaken.

R. P. C. - 2 Analysis of NIH Program Activities SERIAL NO. NMI- 21c
December 1955

Budget Data Sheet

10.

11. BUDGET DATA:

ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
	PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	

12. BUDGET ACTIVITY:

Research

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATION PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 OR 1957:

Student Health Service, Howard University

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

This project supplements and is correlated with research done in the Laboratory of Tropical Diseases, National Microbiological Institute.

Honors, Awards, and Publications Sheet

15.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

None

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

None

R. F. C. - 1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

1. National Microbiological Institute
INSTITUTE

2. Laboratory of Clinical Investigation
LABORATORY OR BRANCH

3. None
SECTION

4. Bethesda, Maryland

5. NMI- 22C
SERIAL No.

6. Infestations due to cestodes (tapeworms)
PROJECT TITLE

7. Dr. Henry K. Beye
PRINCIPAL INVESTIGATOR

8. Dr. Donald E. Kayhoe and Miss Elizabeth Guinn
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives:

To determine the extent of infestations with Cestodes among specific population groups, such as the American Indian, by laboratory and immunological tests; to study clinically selected or referred patients; to evaluate therapeutic regimens.

T. saginata (beef tape worm) and T. solium (pork tape worm) are of principal interest in this category in infestations due to the adult or the larval forms of these species (cysticerosis).

In infestations due to the adults, studies are centered chiefly around the altered physiology of the bowel and therapeutic regimens directed toward eradication of the worm.

In diseases produced by the larvae, the clinical investigative pattern will be influenced greatly by the organs or system chiefly involved. In general, efforts directed twoard a therapeutic agent capable of adversely affecting the larvae (e.g., hetrazan) used adjunctively with an agent tending to reduce the local inflammatory reagent injurious to the host, (e.g., corticosteroids).

In the general category, other species are included, H. diminuta, H. nava; and E. granulosis.

Project Description Sheet

Methods Employed:

Field surveys of selected groups of people as indicated, parasitic disease workup of patients referred by private physicians or governmental organizations for suspected cestode infestations, laboratory study, immunologic techniques, and experimental therapy.

Patient Material:

		No.	Average Stay Days
Admissions:	Adults	0	0
	Children	0	0
Outpatient:	Number of patients	1	
	Number of visits	5	

Major Findings:

No major findings to report because of lack of sufficient patient material.

Significance to Microbiological Research:

Infestations with T. saginata and T. solium often are difficult therapeutic problems, and are not uncommon in the United States; and information on treatment regimens would be welcomed by many practicing physicians. Cases of Echinococcus granulosa, especially among Eskimos, present diagnostic and therapeutic problems which, because of increasing work in tuberculosis among these people, are assuming a great importance. A similar situation will probably be found to exist among the Indian population for which the Public Health Service has assumed responsibility in preventive and curative medicine.

Proposed Course of Project:

If time, staff and relation can be established to initiate survey among Indian population, clinical studies will be determined by availability of patient material.

R. P. C. - 2
December 1955

Analysis of NIH Program Activities SERIAL NO. NMI- 22C

Budget Data Sheet

10.

11. BUDGET DATA:

ESTIMATED	BUDGET POSITIONS			MAN YEARS			PATIENT
EXPENDITURES	PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	DAYS

12. BUDGET ACTIVITY:

Research

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 OR 1957:

None

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

This project supplements and is correlated with research done in the Laboratory of Tropical Diseases, National Microbiological Institute.

Honors, Awards, and Publications Sheet

15.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

None

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

None

R. P. C. - 1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

1. National Microbiological Institute
INSTITUTE

2. Laboratory of Clinical Investigation
LABORATORY OR BRANCH

3. None
SECTION

4. Bethesda, Maryland
LOCATION

5. NMI- 23C
SERIAL NO.

6. Toxoplasmosis
PROJECT TITLE

7. Dr. Henry K. Beye
PRINCIPAL INVESTIGATOR

8. Dr. Donald E. Kayhoe and Dr. Henry G. Cramblett
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives:

To clarify the natural ecology of the disease; to correlate clinical manifestations and pathogenesis; to evaluate diagnostic methods; to determine effective methods of therapy.

Methods Employed:

Serologic surveys; clinical study and observation; work-up of patients referred by private physicians or governmental organizations as suspected cases of toxoplasmosis; laboratory and immunologic diagnostic investigations; and experimental therapy.

Patient Material:

		No.	Average Stay Days
Admissions:	Adult males	2	11
	Adult females	0	0
	Children	0	0
Outpatient:	Number of patients	4	
	Number of visits	16	

Project Description Sheet

Major Findings:

A great deal of previously unknown knowledge was obtained by a detailed study of a case of laboratory acquired adult systemic toxoplasmosis. Data, such as specific incubation period, observations on clinical manifestations, parasitemia, behavior of serum proteins and the changes in their electrophoretic pattern, trends in dye titer and complement fixation reactions, and observations following initiation of "Daraprim" (pyrimethamine) constitute a series of major findings. Two cases have been previously reported, but the present patient is the first one it was possible to study in such detail.

Collaboration with the staff of the National Institute of Neurological Diseases and Blindness continued in the study of cases with uveitis, serological reactions before and after chemotherapy, and clinical responses. No conclusions are yet warranted.

Significance to Microbiological Research:

There is evidence of widespread human exposure and high incidence of immunologic response. It is known to cause blindness and congenital central nervous system changes. The clinical spectrum of the disease, the reliability of diagnostic methods, and the efficacy of specific drugs need clarification.

Proposed Course of Project:

Continue as described; insofar as time and staffing allows, to initiate serologic surveys to discover available people for study, including followup dye titer on patients with negative heterophile titer.

R. P. C. - 2 Analysis of NIH Program Activities SERIAL NO. NMI- 23C
December 1955

Budget Data Sheet

10.

11. BUDGET DATA:

ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
	PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	

12. BUDGET ACTIVITY:

Research

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 OR 1957:

National Institute of Neurological Diseases and Blindness.

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

This project supplements and is correlated with research done in the Laboratory of Tropical Diseases, National Microbiological Institute.

Honors, Awards, and Publications Sheet

15.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

None

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

None

R. P. C. - 1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

1. National Microbiological Institute
INSTITUTE

2. Laboratory of Clinical Investigation
LABORATORY OR BRANCH

3. None
SECTION

4. Bethesda, Maryland
LOCATION

5. NMI- 24C
SERIAL NO.

6. Infectious Mononucleosis
PROJECT TITLE

7. Dr. John P. Utz
PRINCIPAL INVESTIGATOR

8. None
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives:

To assess the degree of involvement especially that in the liver of the human patient with infectious mononucleosis; to attempt virus isolation from patients during various periods of their illnesses using fresh human tissue culture techniques; to determine metabolic and immunologic changes in patients with severe generalized viral disease; to investigate for other than viral causes of this syndrome: Listerellosis, Toxoplasmosis.

Methods Employed:

Hospitalization of patients for study by clinical observation and laboratory methods. Laboratory methods involve primarily the newer tissue culture techniques, such as attempts to unmask viruses by the Huebner-Rowe technique.

Patient Material:

		No.	Average Stay Days
Admissions:	Adult males	0	0
	Adult females	1	29
	Children males	1	79
	Children females	0	0
Outpatient:	Number of patients:	3	
	Number of visits:	9	

Analysis of NIH Program Activities SERIAL NO. NMI-24C

Project Description Sheet

Major Findings:

None. Sufficient patient material has not been available.

Significance to Microbiological Research:

Infectious mononucleosis is a common and perhaps even universal infection of man. Certain characteristics of the disease suggest that it may be an intermediate link between infections and neoplastic blood dyscrasias. Since heterophile titers develop, the disease lends itself to basic studies in immunology.

Proposed Course of Project:

This project will be pursued to the extent allowed by availability of patient material.

R. P. C. - 2 Analysis of NIH Program Activities SERIAL NO. NMI-24C
December 1955

Budget Data Sheet

10.

11. BUDGET DATA:

ESTIMATED	BUDGETED POSITIONS			MAN YEARS			PATIENT
EXPENDITURES	PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	DAYS

12. BUDGET ACTIVITY:

Research

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 OR 1957:

None

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

This project supplements and is correlated with research done in the Laboratory of Infectious Diseases, National Microbiological Institute.

Honors, Awards, and Publications Sheet

15.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

None

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

None

R. F. C. - 1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

1. National Microbiological Institute
INSTITUTE

2. Laboratory of Clinical Investigation
LABORATORY OR BRANCH

3. None
SECTION

4. Bethesda, Maryland
LOCATION

5. NMI- 25C
SERIAL NO.

6. Rheumatic Fever
PROJECT TITLE

7. Dr. John P. Utz
PRINCIPAL INVESTIGATOR

8. Dr. Robert H. Parrott
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives:

To evaluate and establish clinical and laboratory criteria for diagnosis. To study pathogenesis of the disease, particularly the factors that lead to cardiac involvement, and experimental therapy.

Methods Employed:

Clinical studies. Evaluation of various fractions of streptococci and their corresponding antibodies as immunologic aids in diagnosis. Correlation of various biochemical tests with clinical observations.

Patient Material:

		No.	Average Stay Days
Admissions:	Adult males	3	55
	Adult females	1	12
	Children males	2	68
	Children females	7	58
Outpatient:	Number of patients:	19	
	Number of visits:	228	

Major Findings:

Series too small for evaluation.

Significance to Microbiological Research:

Rheumatic fever is a major unsolved public health problem. Aids to definitive diagnosis are badly needed.

Proposed Course of Project:

This project will be pursued if and when patient material becomes available.

R. P. C. - 2
December 1955

Analysis of NIH Program Activities

SERIAL NO. NMI-25C

Budget Data Sheet

10.

11. BUDGET DATA:

ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
	PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	

12. BUDGET ACTIVITY:

Research

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL, FOR THIS PROJECT IN EITHER 1956 OR 1957:

None

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

This project supplements and is correlated with research done in the Laboratory of Infectious Diseases, National Microbiological Institute.

Honors, Awards, and Publications Sheet

15.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

None

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

None

R.P.C. - 1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

1. National Microbiological Institute
INSTITUTE

2. Laboratory of Clinical Investigation
LABORATORY OR BRANCH

3. None
SECTION

4. Bethesda, Maryland
LOCATION

5. NMI- 266
SERIAL NO.

6. Leptospirosis
PROJECT TITLE

7. Dr. Norman B. McCullough
PRINCIPAL INVESTIGATOR

8. Dr. John P. Utz
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives:

Studies are directed toward evaluation and improvement of methods of diagnosis, toward determining the clinical course and complications and sequellae due to the more recently recognized species of Leptospira, in particular *L. pomona*. Therapeutic agents will be evaluated.

Methods Employed:

Clinical study of patients; experimental therapy; laboratory diagnostic techniques, epidemiological surveys on occupational groups; etiologic surveys on patients with aseptic meningitis.

Patient Material:		No.	Average Stay Days
Admissions:	Adults	0	0
	Children	0	0
Outpatient:	Number of patients	5	
	Number of visits	10	

Major Findings:

This project will become active when patient material becomes available. Some limited serological surveys have been made. Approximately 7.5% of the federal meat inspectors in New York City, Baltimore and Chicago were shown to have serological evidence of past infection with L. pomona.

Significance to Microbiological Research:

Leptospirosis is widespread in our domestic animals. The extent of the problem in man and reliability of diagnostic tests needs definition, as does the clinical spectrum of the disease. No effective therapy is known.

Proposed Course of Project:

Continue as outlined above.

R. P. C. - 2 Analysis of NIH Program Activities SERIAL NO. NMI- 23C
December 1955

Budget Data Sheet

10.

11. BUDGET DATA:

ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
	PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	

12. BUDGET ACTIVITY:

Research

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 OR 1957:

None

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

This project supplements and is correlated with research done in the Laboratory of Tropical Diseases, National Microbiological Institute.

R.P.C. - 3 Analysis of NIH Program Activities Serial No. NMI-126C
December 1955

Honors, Awards, and Publications Sheet

15.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

None

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

None

R. P. C. - 1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

1. National Microbiological Institute
INSTITUTE

2. Laboratory of Clinical Investigation
LABORATORY OR BRANCH

3. None
SECTION

4. Bethesda, Maryland
LOCATION

5. NMI- 27C
SERIAL NO.

6. Streptococcal Disease: a. Glomerulonephritis b. Subacute bacterial endocarditis
PROJECT TITLE

7. Dr. John P. Utz
PRINCIPAL INVESTIGATOR

8. Dr. Norman B. McCullough and Dr. Robert H. Parrott
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives:

To investigate the pathogenesis of acute diffuse glomerulonephritis. To determine the course and sequellae of this disease in children. To establish guidelines for therapy. To establish guidelines for the antibiotic therapy of subacute bacterial endocarditis.

Methods Employed:

Glomerulonephritis Clinical Studies: Immunologic studies employing various streptococcal derivatives in standard laboratory tests; the application of tissue culture techniques to cell-antigen systems possibly operative in this disease; determination of antikidney-protein antibodies in serum of patients; and correlation of results with history and clinical course.

Subacute bacterial endocarditis: Standard laboratory tests for determining antibiotic sensitivities of causative organisms; determining antibiotic sensitivities of causative organisms; determination of antibiotic serum levels; use of various antibiotic regimens.

Patient Material:

		No.	Average Stay Days
Admissions:	Adult males	4	5
	Adult females	1	13
	Children males	1	124
	Children females	0	0
Outpatient:	Number of patients	17	
	Number of visits	204	

Project Description Sheet

Major Findings:

Series is too small for definite conclusions.

Significance to Microbiological Research:

The pathogenesis of glomerulonephritis is unknown. The disease is common and sequellae are serious. The disease lends itself to the study of basic immunologic phenomena.

Proposed Course of Project:

Dependent upon availability of clinical material.

R. P. C. - 2
December 1955

Analysis of NIH Program Activities

SERIAL NO. NMI- 27C

Budget Data Sheet

10.

11. BUDGET DATA:

ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
	PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	

12. BUDGET ACTIVITY:

Research

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 OR 1957:

None

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS) IDENTIFY SUCH RESEARCH:

This project supplements and is correlated with research done in the Laboratory of Infectious Diseases, National Microbiological Institute.

Analysis of NIH Program Activities SERIAL NO. NMI- 27C

Honors, Awards, and Publications Sheet

15.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

None

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

None

R. P. C. - 1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

1. National Microbiological Institute
INSTITUTE

2. Laboratory of Clinical Investigation
LABORATORY OR BRANCH

3. None
SECTION

4. Bethesda, Maryland
LOCATION

5. NMI- 28C
SERIAL NO.

6. Trematode Infestations
PROJECT TITLE

7. Dr. Henry K. Beye
PRINCIPAL INVESTIGATOR

8. Dr. Donald E. Kayhoe and Miss Elizabeth Guinn
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives:

A. Schistosomiasis

B. Other Fluke diseases

Besides schistosomiasis, this project includes paragonimiasis and other fluke diseases.

To elucidate systemic reactions to the infection, immunologic responses, and pathologic reactions in the organ or system particularly affected; to evaluate new or experimental prophylactic or therapeutic drugs; to improve diagnostic techniques.

Methods Employed:

Field surveys of selected groups of people (Puerto Ricans) for trematode infestations; parasitic disease work-up of patients referred by private physicians or governmental organization for suspected trematode infestations; parasitological methods of diagnosis applied to feces, urine, bile, etc; serologic studies, including appropriate skin tests, complement-fixation reactions, and reactions between ova, and patient serum; clinical studies on proved cases; experimental therapy.

Analysis of NIH Program Activities SERIAL NO. NMI- 28C

Project Description Sheet

Patient Material:

		No.	Average Stay Days
Admissions:	Adults	0	0
	Children	0	0
Outpatient:	Number of patients	2	
	Number of visits	6	

Major Findings:

The discovery of one student having schistosomiasis, in a pilot survey for intestinal parasites among 33 foreign students at Howard University, indicates there might be sufficient available patient material in the Washington area for an earlier development of this project than anticipated.

The number of patients studied is still too small to warrant conclusions.

Significance to Microbiological Research:

The great immigration of infected Puerto Ricans into the United States, especially the New York area; the severity and importance of schistosomiasis as a major disease in most parts of the world; the fact that the disease has not yet been controlled in endemic areas; the realization that the United States is spending many hundreds of millions of dollars to increase economic productivity in these endemic areas; and the knowledge that many aspects of the disease are little known, emphasizes the significance of the National Microbiological Institute research interest.

Proposed Course of Project:

As time and staffing allows, to instigate surveys among groups of people coming to the United States from endemic areas. Proceed as described with selected cases.

R. P. C. - 2 Analysis of NIH Program Activities SERIAL NO. NMI-28C
December 1955

Budget Data Sheet

10.

11. BUDGET DATA:

ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
	PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	

12. BUDGET ACTIVITY:

Research

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957:

Foreign Student Service, Howard University, Washington, D.C.

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

This project supplements and is correlated with research done in the Laboratory of Tropical Diseases, National Microbiological Institute.

Honors, Awards, and Publications Sheet

15.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

None

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

None

R. P. C. - 1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

1. National Microbiological Institute
INSTITUTE

2. Laboratory of Clinical Investigation
LABORATORY OR BRANCH

3. None
SECTION

4. Bethesda, Maryland
LOCATION

5. NMI- 29C
SERIAL NO.

6. Brucellosis
PROJECT TITLE

7. Dr. Norman B. McCullough
PRINCIPAL INVESTIGATOR

8. Dr. Thomas L. Gorsuch
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives:

To study the means of diagnosing the disease in humans, especially in borderline cases of chronic illnesses in which present cultural and serologic techniques fail to provide reliable evidence of infection; to study the pathogenesis of the disease especially in regard to liver involvement; to evaluate treatment; and to study metabolic changes in patients with severe febrile illnesses.

Methods Employed:

Clinical study of patients; extensive laboratory studies aimed at cultural proof of brucellosis; serial measurements of liver function; antibody production, electrophoretic patterns of sera, and blood antibody levels.

Patient Material:

		No.	Average Stay Days
Admissions:	Adult males	19	36
	Adult females	3	19
	Children	0	0
Outpatients:	Number of patients:	45	
	Number of visits:	180	

Project Description Sheet

Major Findings:

The number of patients studied is too small to yield conclusive results. Accumulated data continues to suggest that most patients with vaguely defined illness and low Brucella agglutinin titer do not have brucellosis.

Evaluation of therapy is hindered by lack of patient material.

Significance to Microbiological Research:

Illustrates attention directed toward a disease in man and animals of major economic importance; a disease for which no satisfactory treatment has been available until recent years when combined antibiotic therapy began to be used and to be evaluated by studies such as this. Thirdly, attention is being directed toward major responses of patients to severe inflammatory disease which responses may be common to many types of illness.

Proposed Course of Project:

Continue as outlined.

R. P. C. - 2 Analysis of NIH Program Activities SERIAL NO. NMI- 290
December 1955

Budget Data Sheet

10.

11. BUDGET DATA:

ESTIMATED EXPENDITURES	BUDGETED POSITIONS			MAN YEARS			PATIENT DAYS
	PROF	OTHER	TOTAL	PROF	OTHER	TOTAL	

12. BUDGET ACTIVITY:

None

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 OR 1957:

Bureau of Animal Industry, U. S. Department of Agriculture.

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

This project supplements and is correlated with the brucellosis project of the Laboratory of Infectious Diseases, NMI, which is directed by the same principal investigator.

Honors, Awards, and Publications Sheet

15.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

(1) McCullough, Norman B. : Brucellosis in Children. The Pediatric Clinics of North America, 2(1):, February, 1955.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955.

None

R. P. C. - 1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

1. National Microbiological Institute
INSTITUTE

2. Laboratory of Infectious Diseases
LABORATORY OR BRANCH

3. Office of the Chief
SECTION — UNIT

4. Bethesda
LOCATION

5. NMI - 30
SERIAL No.

6. Office of the Chief
PROJECT TITLE

7. Dr. Dorland J. Davis
PRINCIPAL INVESTIGATOR

8. Dr. Roger M. Cole, Asst. to the Chief
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Office of the Chief

The responsibilities of the Office of the Chief include the maintenance and encouragement of an atmosphere conducive to productive original investigation in the field of infectious diseases. This office must be responsive to the needs in this field, the developments of new methods and knowledge, and the capabilities of individual scientists. The responsibilities also include the selection and recruitment of the most promising investigators when required and the selection of competent subprofessional personnel. In addition this office performs administrative duties designed to facilitate research efforts by its scientists.

The organization of the laboratory has remained essentially the same as in previous years and two more investigators have joined the research staff. Because of the demand for space to house the increased activities of the Division of Biologics Standards, a number of laboratory rooms were lost to the Laboratory of Infectious Diseases which required the transfer of one research unit to the Rocky Mountain Laboratory and curtailment of space for other units.

A series of seminars was conducted by investigators in the laboratory and also by scientists from other research institutions. These pertained to problems of interest to the staff and many concerned studies on basic immunologic problems.

At the time of the delay in distribution of poliomyelitis vaccine in April and May, the laboratories of the Sectional

Research Program were asked to assist in specific diagnosis of poliomyelitis cases among vaccinated individuals or suspected cases. Twenty-one laboratories which are located in private research centers, universities, hospitals and state health departments willingly agreed to participate and contracts were negotiated with each one for diagnostic services. This aspect of the study greatly augmented the information available to the Poliomyelitis Surveillance Unit of the Communicable Disease Center in its effort to follow the incidence of the disease.

Because the objectives of the Sectional Research Program have shifted from a research emphasis to development of training, improved communications, and evaluation of diagnostic procedures, the Program was transferred to the Laboratory Branch, Communicable Disease Center in October.

Budget Data Sheet

10.

11.

12. BUDGET ACTIVITY:

Research

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATION PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957.

None

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PHS (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS) IDENTIFY SUCH RESEARCH.

None

Honors, Awards, and Publications Sheet

15.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955

Davis, Dorland J.: Psittacosis in Pigeons, Chapter 7, Textbook entitled PSITTACOSIS, DIAGNOSIS, EPIDEMIOLOGY, AND CONTROL. Edited by F. R. Beaudette, Rutgers University Press, New Brunswick, N. J., 1955.

Davis, Dorland J.; Pittman, Margaret; Vogel, John E.; and Ottinger, Barbara: Susceptibility of Haemophilus aegyptius to Antibiotics in Embryonated Eggs and in Artificial Media. Antibiotics and Chemotherapy, Vol. 5, No. 7, 363-369, July 1955.

Pittman, Margaret and Davis, Dorland J.: Ineffectiveness of Penicillin, Rivanol Lactate, and Tyrothricin on the Development of Resistance of Haemophilus aegyptius to Streptomycin. Antibiotics and Chemotherapy, Vol. 5, No. 7, 370-374, July 1955.

17. HONORS OR AWARDS FOR THIS PROJECT

Dorland J. Davis - Recipient Edward Rhodes Stitt Award, 1955, from the Association of Military Surgeons.

R. P. C. - 1 Analysis of NIH Program Activities
December 1955

Project Description Sheet

1. National Microbiological Institute
INSTITUTE

2. Laboratory of Infectious Diseases
LABORATORY OR BRANCH

3. Bacterial & Mycotic Diseases
SECTION

4. Bethesda, Maryland
LOCATION

5. NMI - 31
SERIAL NO.

6. Investigations on Etiological Agents of Diarrheal Diseases
PROJECT TITLE

7. Dr. A. Elizabeth Verder
PRINCIPAL INVESTIGATOR

8. None
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

A. Investigations on Etiological Agents of Diarrheal Diseases

Objectives:

Investigation of outbreaks of diarrheal disease from the standpoint of etiology and epidemiology; intensive research is carried out on those outbreaks of unknown etiology or unusual mode of spread.

Methods Employed:

Studies are initiated through the use of conventional techniques for the isolation of potentially pathogenic Enterobacteriaceae and other bacterial pathogens known to cause gastro-enteritis. When rigorous application of the techniques does not reveal the etiology, new methods must be developed and tried. In view of recently reported findings not only potentially pathogenic bacteria but also possible viral and rickettsial agents should be sought.

Appropriate serological methods are employed for the identification and typing of the strains of organisms isolated and for the study of the immunological response of the individuals involved in the outbreak to the agent regarded as the probable etiological agent or as a contributing factor in the illness.

Major Findings:

Unpublished data have been accumulated on outbreaks in which organisms of the Arizona group and the Ballerup-Bethesda group of the Enterobacteriaceae have been isolated from the stools of a sufficiently large number of adults, who were involved, to suggest some correlation between their presence and the development of the illness. The significance of these organisms as causative agents remains to be established. The presence of pathogenic viruses or rickettsia was either not investigated or not thoroughly explored.

Significance to Microbiological Research:

Diarrheal diseases, as all other phases of gastroenterology, warrant investigation. The loss of "man-hours" in business and industry as a result of bouts of gastro-enteritis should emphasize the need for research even though mortality rates are low.

The variation in the response of individuals following the ingestion of even large numbers of known enteric pathogens such as strains of Salmonella and our newly acquired knowledge of their ubiquitous distribution raise many pertinent questions and suggest promising lines of research.

Proposed Course of Project:

Unless other units collaborate in the study of outbreaks, the research must be limited to the search for readily detected bacterial agents because of the lack of personnel, laboratory space, equipment, etc.

Under existing circumstances the exploration of the reactions of very young experimental animals to known enteric pathogens, when factors which may influence susceptibility are varied, would be the most logical approach.

B. L-phase Variants and Pleuropneumonia-like Organisms

Objectives:

To study the characteristics of some L-phase variants and pleuropneumonia-like organisms isolated from man and animals.

Methods Employed:

Bacteriological technics will be used to study the morphology and physiology of the organisms including nutritional and growth requirements, growth in tissue culture, biochemical properties, filterability, immunological reactions and pathogenicity.

Major Findings:

Work was started following the isolation of some cultures from humans. Attempts to study the pathogenicity of these strains for laboratory animals led to the isolation of some animal strains. Studies which have been carried out so far have served primarily to point out the many difficulties with which workers with this group are confronted. Since many laboratory animals harbor morphologically similar strains, satisfactory animals for use in studies on pathogenicity are being sought.

Significance to Microbiological Research:

The group of pleuropneumonia-like organisms and the morphologically similar L-phase variants of bacteria have not been studied sufficiently for their importance as infectious agents to be evaluated satisfactorily. Since strains are being isolated in considerable numbers by various workers from both animals and man, their significance as potential pathogens should be appraised.

Proposed Course of Project:

Intensive work on a few strains is proposed so that methods may be obtained for the study of a larger number of strains from various sources.

C. Pathogenicity of Organisms of Genus Pseudomonas

Objectives:

To elucidate the role of organisms of genus Pseudomonas as human pathogens.

Methods Employed:

In vitro studies to detect properties similar to those which characterize known human pathogens of other genera and in vivo work with animals to study their ability to invade tissues and/or their capacity for damaging them.

Major Findings:

Studies on the classification of a large number of strains have given us: 1) biochemical characteristics which permit more accurate separation of the species within the genus as well as identification of an organism as a Pseudomonas and 2) serological types within the large group of Ps. aeruginosa (the most important species involved in production of human illness) so that much more exact identification of strains, isolated from various tissues of an individual and his environment, is possible.

Significance of Microbiological Research:

Ps. aeruginosa appears to take the role of an opportunist and invade tissues when the defense mechanisms are altered. We frequently fail to assess the importance of its role as a pathogen especially in infancy and childhood, in debilitated adults, and following shock even in healthy individuals. The organisms are ubiquitous and are found in large numbers in the environment of hospital patients.

Proposed Course of Project:

Studies on classification will be made available for use by other laboratories. Additional work will be pursued as far as personnel, space and pressure of work on other projects permit.

Budget Data Sheet

10.

11.

12. BUDGET ACTIVITY:

RESEARCH

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATION PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957

Dr. Edwin M. Lerner, Jr., Section on Pathologic Anatomy, NIAMD, has been and will continue to be collaborator on these studies.

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PHS (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS) IDENTIFY SUCH RESEARCH.

CDC projects on Control of Diarrheal Diseases.

R. P. C. - 3 Analysis of NIH Program Activities SERIAL NO. NMI -31

Honors, Awards, and Publications Sheet

15.

16. None

LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

17. None

HONORS OR AWARDS FOR THE PROJECT

R. P. C. - 1 Analysis of NIH Program Activities
December 1955

Project Description Sheet

1. National Microbiological Institute
INSTITUTE

2. Laboratory of Infectious Diseases
LABORATORY OR BRANCH

3. Bacterial & Mycotic Diseases
SECTION

4. Bethesda, Md.
LOCATION

5. NMI - 32
SERIAL NO.

6. Studies of Mycoses and Pathogenic Fungi
PROJECT TITLE

7. Dr. C. W. Emmons
PRINCIPAL INVESTIGATOR

8. Dr. Wm. L. Jellison - Rocky Mountain Laboratory, Hamilton, Montana
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

A. Saprophytic Occurrence of Pathogenic Fungi

Objectives:

To determine sources of human infection, to provide supporting diagnostic evidence in specific cases of mycoses, to obtain basic information concerning the factors which control geographic distribution of pathogens.

Methods:

Specimens of soil and organic debris are collected from indicated sites, injected intraperitoneally into mice, the mice are killed one month later and cultures are made from lesions or from liver and spleen.

Major Findings:

Additional isolations of Histoplasma capsulatum continue to show its frequent saprophytic occurrence in litter and soil from chicken houses. Other pathogens have also been isolated, the most remarkable during the year being the isolation of Cryptococcus neoformans from 63 of 111 specimens of pigeon dung. This laboratory has pioneered in these studies.

Significance:

The demonstration that pathogenic fungi have an independent saprophytic occurrence in soil or organic debris is the essential key to the peculair epidemiology of the mycoses.

Proposed Course:

These studies will be continued with emphasis upon the ecological factors which determine the microdistribution of those pathogenic fungi in soils, and a search for effective control methods which may be applicable in special environments.

B. Field Study of Coccidioidomycosis in Utah

Objective:

To determine the prevalence of coccidioidomycosis and extent of the endemic areas in southern Utah.

Methods:

Collection of rodents, culture of lungs, and blind passage of tissues to mice.

Major Findings:

Study of material is not complete. 480 animals were collected and processed. There are three possible isolations of *Coccidioides immitis*, 2 of *Cryptococcus neoformans*, 4 of *Trichophyton sp.*, 57 of *Haplosporangium* and numerous isolations of *Aspergillus fumigatus* from these rodent lungs.

The study was conducted in collaboration with Utah State Health Department officials who were simultaneously skin testing children and cattle.

Significance:

This is apparently the most northerly extension known of the endemic area of coccidioidomycosis. Previously, information about it had been based only on two isolations of the fungus from rodents by Jellison, and by small scale skin testing studies. Since this appears to be a border-line of the endemic area and is so far north, further field studies could be expected to yield important information concerning the factors which determine the endemicity of the fungus.

Proposed Course:

The future plans for this project await results of the current study of material.

C. Chemotherapy of Experimental Mycoses

Objectives:

To find safe and effective fungicides for eventual clinical use.

Methods:

Mice are infected intravenously with a standardized dose and treatment with drugs is begun on the following day by intraperitoneal injection. Therapy is tested against experimental histoplasmosis and cryptococcosis. The effectiveness of a drug is measured by increased survival time of treated mice and alterations in the slope of the cumulative death curve. In some experiments cultures are made from tissues of surviving mice to detect sterilization of tissues.

Major Findings:

Standardized procedures giving uniform death curves in control groups have been developed. The margin between toxic and therapeutically effective doses of most antifungal antibiotics and other fungicides is too narrow to show dramatic results. Ascosin, candicidin, mycostatin, and eulicin show some promise. The most important finding has been an unexpected favorable effect of various silver salts on the course of cryptococcosis. It is hoped to have this material ready for report in the near future.

Significance:

There is no presently available safe and effective chemotherapy for some of the most important fatal mycoses. In vivo screening of new antifungal agents in experimental mycoses must precede clinical tests.

Proposed Course:

These studies will be continued. Coccidioidomycosis will be added to the list of test mycoses.

D. Laboratory and Clinical Research utilizing Various Mycoses as they become Available

Objectives:

To study clinical variations of mycoses and the effect of chemotherapy in the systemic mycoses. To detect and study new and unusual mycoses.

Methods:

Collaboration with the staff of the Clinical Center in observation of cases and study of pathologic material. Maintenance of a limited diagnostic service to Washington physicians and hospitals and to certain associates abroad for the purpose of obtaining for research purposes rare and unusual mycotic material to our mutual benefit.

Major Findings:

Thorough study of unusual as well as more routine mycoses has been possible in the Clinical Center. These have included a unique case of endocarditis caused by Crptococcus neoformans and cases involving infection by two fungi. Interesting and unusual fungi have been received and identified from local sources and many parts of the world. In collaboration with Dr. Lie (see bibliog.) a unique case of dermal infection with Basidiobolus ranarum has been reported. An equally unusual case from the same collaborator is under study and nearly ready to be reported. Many other interesting but less spectacular cases have come under study.

Significance:

The clinical variations of the mycoses are still imperfectly known and careful case histories often add to our knowledge of a mycosis. Unusual opportunities are offered to correlate studies of experimental therapy and clinical trial. Maintenance of the diagnostic service mentioned above has been highly gratifying in establishing clinical contacts which have brought to the Mycology Unit very valuable research material.

Proposed Course:

Project will be continued.

Budget Data Sheet

10.

11.

12. BUDGET ACTIVITY:

RESEARCH

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATION PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957

Project 9B was conducted in cooperation with Dr. William L. Jellison, Rocky Mountain Laboratory and Mr. E. A. Shippee, Dugway Proving Grounds.
Project 9D was conducted with the cooperation of Dr. Walter Pacosa, Childrens Hospital, D.C.; Dr. A. Symeonidis (formerly in the Cancer Institute), Dr. Lie Kian Joe and associates of Indonesia, and many other physicians.

14. None

IF THIS PROJECT RESEMBLES, COMPLEMENTS OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PHS (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS) IDENTIFY SUCH RESEARCH.

15.

16.

LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

Emmons, C.W. & Rowley, D.A. Isolation of *Histoplasma capsulatum* from fresh and deep-frozen peribronchial lymph nodes of dogs by mouse inoculation. J. Lab. Clin. Med. 45(2):303-307. Feb. 1955.

Emmons, C.W., Rowley, D.A., Olson, B.J., Mattern, C.F., Bell, J.A., Powell, E. & Marcey, E.A. Histoplasmosis. Proved occurrence of inapparent infection in dogs, cats and other animals. Am. J. Hyg. 61(1): 40-44, Jan. 1955.

Emmons, C.W. Saprophytic sources of *Cryptococcus neoformans* associated with the pigeon (*Columba livia*). Am. J. Hyg. 62( ): 227-232, Nov. 1955.

Symeonidis, A. & Emmons, C.W. Granulomatous growth induced in mice by Absidia corymbifera. AMA Arch. Path. 60:251-258. Sept. 1955.

Pacosa, W. & Emmons, C.W. Histoplasmosis. Clin. Proc. Childrens Hospital, Washington, D.C. 11(5): 95-102, May 1955.

Rowley, D.A. & Huber, M. Pathogenesis of experimental histoplasmosis in mice. I. Measurement of infecting dosages of the yeast phase of *Histoplasma capsulatum*. J. Inf. Dis. 96:174-183. 1955.

Rowley, D.A. & Huber, M. Pathogenesis of experimental histoplasmosis in mice. II. Comparison of the intravenous and intraperitoneal routes of infection. J. Infect. Dis. 97:27-34, 1955.

In Press.

Emmons, C.W. The isolation of *Cryptococcus neoformans* from pigeon dung. Proc. Symposium on Fungus Diseases, Los. Angeles, June 23, 1955.

Emmons, C. W. Medical Mycology. Chapter in "Clinical Laboratory Methods and Diagnosis", Ed. 4 by R.B.H. Gradwohl.

Emmons, C. W. Mycoses of Animals. Chapter in "Advances in Veterinary Science. Vol. 2.

Lie, K. J., Njo-Injo, T.E., Pohan, A., van der Meulen, H. & Emmons, C.W. *Basidiobolus ranarum* as a cause of subcutaneous mycosis in Indonesia. AMA Arch. Dermatol.

Rowley, D.A. & Huber, M. Growth of *Histoplasma capsulatum* in normal, superinfected and immunized mice. J. Immunol.

17. None
HONORS OR AWARDS FOR THE PROJECT

R. P. C. - 1 Analysis of NIH Program Activities
December 1955

Project Description Sheet

1. National Microbiological Institute
INSTITUTE

2. Laboratory of Infectious Diseases
LABORATORY OR BRANCH

3. Bacterial & Mycotic Diseases
SECTION

4. Bethesda, Md.
LOCATION

5. NMI -33
SERIAL NO.

6. Biochemical Studies of Pathogenic Fungi
PROJECT TITLE

7. Dr. Leo Pine
PRINCIPAL INVESTIGATOR

8. Dr. Arden Howell - NIDR
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

A. Studies of the Growth and Metabolism of Microaerophilic and Facultative Fungi

Objectives:

To develop a defined medium which can maintain satisfactory growth of strains of Actinomyces and to compare and study the metabolic systems in several species of Actinomyces.

Methods:

General bacteriological and biochemical procedures supported by specific determinations of growth using optical density, acid formation, gasometric and Warburg procedures, fermentation, gas and enzymatic analyses, chromatography and radioactive tracer studies.

Major Findings:

A defined medium for growth of Actinomyces was developed. For the first time strains of Actinomyces can be cultivated easily and with relatively little danger of morphologic change or loss of cultures. The medium and technics developed for the growth of certain strains allow a clear distinction to be made between facultative and microaerophilic strains. Results emphasize the close relationship between Cohnistreptothrix (Lactobacillus) bifidus and C. (Actinomyces) israeli or C. naeslundi but suggest the following differentiation. C. israeli and C. naeslundi reduce nitrates whereas C. bifidus does not. Both groups ferment glucose with fixation of CO_2 to form lactic, succinic, acetic and formic acids. However, *C. bifidus* forms from 5 to 10 times more acetic acid than do either *C. naeslundi* or *C. israeli*. Growth of *C. bifid* cultures is best supported by lactose or glucose, whereas, growth of *C. israeli* and *C. naeslundi* strains is best on glucose or maltose. Whereas, *C. bifidus* is heterolactic, *C. israeli* and *C. naeslundi* are essentially homolactic. The latter organisms form L (+) lactic acid, apparently with traces of D(-) lactic acid, while the former forms L+.

Significance:

The organisms known as Actinomyces have been difficult to maintain indefinitely in the laboratory. The above medium now permits easy maintenance and cultivation and will facilitate determination of growth factors required. The results emphasize the need for further species differentiation in the genus Actinomyces and furnish means and some descriptive data not previously available for doing this.

Proposed Course:

Tracer and general metabolic studies comparing the metabolism and mechanism of carbon dioxide fixation of *C. bifidus* and *C. israeli* will be continued.

5. Studies of the Growth and Conversion from Mycelium to Yeast of *Histoplasma capsulatum*.

Objective:

To determine the factors influencing the growth and conversion of *H. capsulatum*.

Methods:

Use of a chemically defined basal medium to which additions or deletions of known factors are made, optical density determinations of growth with correlated microscopic observations of phase of growth. Observation of development, progress and phase of experimental infections at two temperatures in the toad, *Bufo marinus*. Experiments supplemented with biochemical procedures involved in chemical synthesis, purification and assay of growth factors.

Major Findings:

Observations of the effect of temperature on the development of experimental histoplasmosis in the toad show that the viability of the yeast phase of Histoplasma is greater at 25 C than at 37 C, that conversion of mycelial to yeast can occur at 25 C in the toad. A maximal synthetic medium for growth of H. capsulatum having some 37 known growth factors supports continued growth of 11 strains in the yeast phase at 37 C and of several strains in the yeast phase at 25 C. Screening of the factors required for growth have indicated that hemin CO_2, thiamine, riboflavin, inositol, nicotinamide, oleic acid, biotin, thioctic acid, coenzyme A, and Ca pantothenate are required or are stimulatory to the growth of some of the 11 strains under specified conditions. Media lacking the vitamins listed above support mycelial but not yeast phase growth.

Significance:

The results emphasize the effect of nutrition as being the primary requirement to induce yeast growth in vivo and in vitro and show that the higher temperature tested is inhibitory to the development of histoplasmosis in the toad. The results suggest the possible use of specific vitamin competitors for in vivo control of the pathogen. The media developed should have wide application as an assay medium for agents toxic to the yeast phase. It will also allow a more precise determination of the factors involved in the interconversion of the yeast and mycelial phases.

Proposed Course:

The project will be continued with a view to determining the amino acid and vitamin requirements of each phase at 25 C and 37 C and comparing metabolic rates under various conditions of phase and temperature.

Budget Data Sheet

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12. BUDGET ACTIVITY:

RESEARCH

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATION PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957

Saprophytic Occurrence of Pathogenic Fungi in collaboration with Dr. Arden Howell, Dental Institute

14. None

IF THIS PROJECT RESEMBLES, COMPLEMENTS OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PHS (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS) IDENTIFY SUCH RESEARCH.

15.

16.

LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

Pine, Leo. 1955 Studies on the growth of *Histoplasma capsulatum*. II. Growth of the yeast phase on agar media. J. Bacteriol. 70, 375-381.

Pine, Leo and Peacock, Carl L. 1955 Reaction of Fumaric Acid with Cysteine. J. Am. Chem. Soc., *77*, 3153.

Rowley, D. A. and Pine, Leo. 1955 Some nutritional factors influencing growth of yeast cells of *Histoplasma capsulatum* to mycelial colonies. J. Bacteriol., 69, 695-700.

Howell, Arden, Jr.[1] and Pine, Leo. 1955 Studies on the growth of species of *Actinomyces*. I. Cultivation in a synthetic medium with starch. J. Bacteriol., *70*, in press.

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[1] National Institute of Dental Research.

Analysis of NIH Program Activities

R. P. C. - 1
December 1955

Project Description Sheet

1. National Microbiological Institute
INSTITUTE

2. Laboratory of Infectious Diseases
LABORATORY OR BRANCH

3. Bacterial & Mycotic Diseases
SECTION

4. Bethesda, Md.
LOCATION

5. NMI - 34
SERIAL NO.

6. Studies on Tuberculosis
PROJECT TITLE

7. Dr. B. J. Olson
PRINCIPAL INVESTIGATOR

8. Dr. G. W. Lones, Dr. B. Prescott, Miss Gladys Kauffman, and Mr. J. Webb
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

A. Development of an Effective Killed Vaccine Against Tuberculosis.

Objectives:

This unit demonstrated that ultra violet killed tubercle bacilli (human virulent) could produce as much protection against tuberculosis as live BCG. The further development of this u.v. killed vaccine for human use is a primary objective with emphasis on efforts to isolate the effective antigen.

Methods:

The basic problem in the further development of a vaccine for tuberculosis is to quantitate the antigenicity of one vaccine as compared to another where both have a degree of effectiveness.

The methods currently employed are all inadequate. Therefore intensive efforts have been made to improve the methods of:

1. Determination of viable numbers of tubercle bacilli in the challenge dose.
2. Routes of infection to give consistant mortality curves.
3. Dosages of antigen in respect to quantity and time of administration.
4. Detection of presence or absence of viable organisms after irradiation.

Major Findings:

None of our available methods of testing antigenicity give consistant enough results as to permit definite quantitation of antigenicity further than demonstration of its presence or absence. Relative antigenicity can only be determined where all materials under study are included in a given experiment. Progress has been made on points 1, 2 and 4 towards effective standardization. Point 3 remains relatively unsolved.

Significance to Microbiological Research:

This is the most important problem in tuberculosis. Until adequate methods to quantitate the antigenicity of a vaccine in a reproducible manner wherever used, are developed, research in this field is necessarily limited to laborious, time consuming and expensive experiments.

Proposed Course of Project:

Continue our efforts.

B. Origination of Methods to Fractionate the Tubercle Bacillus to Yield Fractions as Nearly Unchanged from their Native State, as Possible.

Objectives:

1) The antigenicity of u.v. killed virulent human tubercle bacilli was demonstrated in the tuberculosis unit and subsequently confirmed by other investigators. The objective of the fractionation is to attempt to isolate and evaluate the active antigenic fractions for possible use in human vaccination and immunological diagnosis.

2) The role of the phospholipids and of the waxes as adjuvants for not only tubercle bacilli but other antigens and organisms is being studied as the above fractionations are carried out.

Methods:

Methods employed for this purpose in other bacteria such as, for example, pneumococci were initially utilized and adapted to tubercle bacilli. It became obvious that new methods would have to be developed because of many unusual chemical characteristics of the mycobacteriums, B,B-dihydroxyethylether and propyleneglycol extraction yielded lipopolysaccharides, with methyl carbitol and methylcellosolve extraction lipids in highly purified state were attained.

The chemical differences of pellicle and diffuse growth required a basic standardization of growth procedure. Further study of infra red analyses of isolated polysaccharides of various strains were made.

Major Findings:

The lipo polysaccharides isolated showed serological activity. The lipids isolated by the new solvents were of sufficient purity to permit current immunological studies. The development of standardized conditions of diffuse growth with relatively constant viable numbers showed 18-20% carbohydrates, 8% lipids and 6% nitrogen in 6-8 days. Infra red analyses of polysaccharide showed human, bovine, avian and vole mycobacterium containing a common polysaccharide fraction which is serologically active and immunologically related.

Significance to Microbiological Research:

The need for an effective antigen in tuberculosis is obvious from our current morbidity and mortality statistics. The last antigen developed, prior to U.V. antigen, was BCG over 30 years ago. BCG is admittedly an inadequate means of protection, it merely is the existing extensively tried antigen.

Proposed Course of Project:

This long term project receives increased emphasis this year because of antigenic components available for testing, as space becomes available for animal testing.

C. Studies of the Localization of Radioactive "Trace" Elements in Pulmonary Lesions, including Tuberculosis, utilizing initially Iodine and Fluorine

Objectives:

The object is to determine the extent to which trace elements play an important role in the evolution of pulmonary lesions.

Methods:

Radioactive tracer techniques for determination of quantity of trace element uptake in various stages of the pulmonary and systemic disease.

Major Findings:

The conditions and extent of uptake of radioactive iodine have been determined and quantitated. An unexpected finding was marked shifts in iodine retention on or about the 14th day of the disease.

Significance to Microbiological Research:

The use of radioactive "trace" elements offer a method of evaluating the role played of certain of these elements in the evolution of the tuberculous lesion.

Proposed Course of Project:

The basic knowledge and techniques learned with iodine offer the necessary foundation to the studies of fluorine currently planned. The mechanism of the "14th day rise" in radioactive iodine is under current study.

D. Study of the Factors Influencing the Development of Tuberculosis lesions in the Lungs and their Localization.

Objectives:

The pathogenesis of tuberculosis of the lungs is little understood. The factors determining the rate of progress or arrest of established lesions are largely based on clinical impressions. The studies of this unit using surgical techniques to alter the pulmonary circulation showed profound effects on experimental pulmonary tuberculosis in monkeys. This observation opened up a new experimental approach to a study of pulmonary tuberculosis.

Methods:

Surgical alteration of pulmonary circulation using a modified Blalock procedure, by Drs. Scott and Hanlon for this work. To further investigate the mechanism of the observed changes, it was necessary to use non-infective material. Silica was chosen for this work because of its known ability to produce pulmonary lesions and further, because of the observation of the Saranac group that BCG given to silicotic guinea pigs caused progressive tuberculosis.

Major Findings:

The marked differences of elasticity and gross pathology between the lung with altered circulation and the opposite lung in animals operated prior to exposure suggested further study of the effort of altering the circulations in lungs of animals with already established silicosis. The results of this long term study are not complete.

Significance to Microbiological Research:

The effect of alteration of blood supply to the lungs has a profound effect on the reactivity of the pulmonary tissue to injurious agents. It suggests the observed effects in experimental tuberculosis were at least partly due to a changed reactivity of pulmonary tissue.

Proposed Course of Project:

The preliminary results on BCG in silicotic animals needs further expansion in numbers of animals because of its significance to public health procedure.

The observations of the effect of an alteration in extent of silicosis because of changed pulmonary circulation is a new observation offering certain therapeutic implications. An initial presentation of silica results was presented in October. Due to curtailment of space, this project in respect to silica cannot be further studied.

Budget Data Sheet

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BUDGET ACTIVITY:

RESEARCH

13.

IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATION PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957

Parke, Davis & Co., collaborating on UV vaccine production.
NHI, Vanderbilt and St. Louis Universities collaborating on surgical procedures in experimental tuberculosis in experimental animals.
NDI and Radio Isotope Lab., NIH collaborating on fluorides in experimental tuberculosis.
Environmental Health Center, BSS,PHS collaborating on study of chemical antigens of tubercle bacilli.

14. None

IF THIS PROJECT RESEMBLES, COMPLEMENTS OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PHS (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS) IDENTIFY SUCH RESEARCH.

Honors, Awards, and Publications Sheet

15.

16.

LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

EMMONS, C. W., Rowley, D. A., Olson, B. J., Mattern, C. F., Bell, J. A., Powell, E. and Marcey, E. A. Histoplasmosis: Proved occurrence of inapparent infection in dogs, cats and other animals. Am. J. Hyg. 61(1): 40-44, Jan. 1955.

In Press:

JOHNS, Thomas N. P. and Olson, Byron J. "Experimental Studies of Temporary Coronary Occlusion".

SCOTT, H. William, Jr., Hanlon, C. Rollins, Olson, Byron J., Fite, George L., Mattern, C.F.T. "Experimental Silicosis: Changes in the Disease in the Monkey Produced by Arterializing the Lung".

MATTERN, Carl F. T., Brackett, Frederick S. and Olson, Byron J. "Appraisal of New Instrument for Counting Particles by Electrical Gating". I. Blood Cell Counting.

17. None

HONORS OR AWARDS FOR THIS PROJECT

R. P. C. - 1 Analysis of NIH Program Activities
December 1955

Project Description Sheet

1. National Microbiological Institute
INSTITUTE

2. Laboratory of Infectious Diseases
LABORATORY OR BRANCH

3. Bacterial & Mycotic Diseases
SECTION

4. Bethesda, Maryland
LOCATION

5. NMI - 35
SERIAL NO.

6. Studies on Chemistry of Tuberculosis. Completion of Immunological Studies on Pneumococci
PROJECT TITLE

7. Dr. B. Prescott and Miss Gladys Kauffmann
PRINCIPAL INVESTIGATOR

8. Dr. B. J. Olson
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

A. Study of Toxicity and Detoxification of Cycloserine, a New Antibiotic Therapeutically Effective in Human Tuberculosis; and Methods of Assay in Blood Plasma.

Objectives:

To determine the toxicity in DBA mice of cycloserine as one of a group of therapeutic agents currently in use in treatment of human tuberculosis; to find means of reducing the toxic action; to find methods for assay of blood plasma levels of cycloserine alone and in mixtures with detoxifying chemicals.

Methods Employed:

Since cycloserine is lethal for mice only in large amounts (5 of 6 mice survived a single oral dose of 200 mg (10 g/kg), the acute toxicity is not the problem. Rather its toxicity is manifested by neuropathologic symptoms. Consequently many chemicals have been administered simultaneously with cycloserine in attempts to reduce the "circling" activity which is always observed with doses of 25 mg per mouse or more.

Major Findings:

Various amino acids, vitamins, metabolites, other antitubercular agents, some alkaloids have been tested orally in both simultaneous and staggered treatments with cycloserine. A few compounds (reserpine and isoniazid, particularly) show promise.

Significance to Microbiological Research:

Greater therapeutic effectiveness against infections with tubercle bacilli may be achieved if dosages of cyclosorine may be increased, either without or with reduced toxic manifestations.

Proposed Course of Project:

The study is being continued on the few compounds that show promise.

B. Development of methods to increase the tolerated dose of isoniazid in experimentally infected animals, and to develop a simple method of determining level in blood, tissues and excreta of animals.

Objectives:

To determine 1) if tuberculous animals could be sterilized in respect to infection and 2) if increasing dosages resulted in the emergence of more resistant strains; 3) and if the organisms were rendered avirulent; 4) evaluate effectiveness of the drug in experimental tuberculosis.

Methods:

The effect of known natural detoxifying agents were studied for the first time in respect to their administration simultaneously with large doses of isoniazid to normal animals and experimentally infected animals.

Major Findings:

(1) The detoxifying chemicals, glycine and sodium glucuronate, 50 mg each, with 10 mg INH per mouse gave 80% survival of 178 - 208 days in normal mice and 210 days in tuberculous mice as contrasted with 41 days in normal and 70 days in tuberculous mice with a 4 mg dose of INH. (Two mice of the 10-50-50 group survived until being sacrificed on the 377th day). (2) With the 10-50-50 dosage, no tubercle bacilli were recovered from either spleen or lung from six of the 24 animals among the 30 on this dosage. This was the only group yielding negative cultures. (3) As to resistant strains, two were recovered from the 4-mg treated group, three from the 10-50-50 group and 8 from the control mice. From the treated animals the resistant strains were recovered from the spleens, whereas 7 of 8 of those from the control mice were from the lungs.

Significance to Microbiological Research:

There is no reason to expect that large dosages of isoniazid long continued will result in more resistant strains appearing nor that larger doses of isoniazid will sterilize an infection. Larger total dosage of isoniazid was given animals than previously possible. Despite this large continued dosage of isoniazid (1) the tuberculous animals were not sterilized of infection (2) the emergence of resistant strains was independent of isoniazid dosage above 2 mgm per 20 gram mouse and (3) strains resistant to 40 mu had unaltered virulence in two instances and one instance of altered virulence. The methods of determining levels of isoniazid has proven applicable to human and animal studies.

Proposed Course of Project:

Study of further methods to increase the tolerated dose of INH in normal animals is in anticipation of application to animals infected with tubercle bacilli. Thus far tests have been made of various amino acids, of vitamins, and of some alpha-keto acids, with best results obtained with respectively cysteine, pyridoxal, and alpha-keto glutaric acid or sodium pyruvate.

C. Study of hydrazine assay in blood plasma and of reducing hydrazine toxicity in mice.

Objectives:

To determine a method of reducing the toxicity of hydrazine, an industrially hazardous chemical; to find a method of hydrazine assay in blood plasma.

Methods Employed:

The effect of certain alpha-keto acids, known metabolites, was studied as prophylactic or therapeutic detoxifying agent for hydrazine in normal mice.

Major Findings:

Prophylactic oral administration of alpha-keto glutaric acid was found to be the most effective procedure for detoxifying hydrazine in DBA mice. Optimum conditions were administration of 0.5 millimol of alpha-keto glutaric acid orally 30 minutes prior to the lethal dose of hydrazine, 100-125 mg/kg. Blood plasma levels were determined by an adaptation of the "pyridyl" method which had been developed for isoniazid (a hydrazine derivative) determination in blood plasma.

Significance to Microbiological Research:

Application of the "pyridyl" test to the assay of free hydrazine in blood plasma was being made while isoniazid toxicity studies were in progress. It was logical to make toxicity and detoxification studies on hydrazine in mice. This information is basic to chemotherapeutic studies. It is further of value in industrial health due to the present extensive use of hydrazine, especially as propellant in jets and rockets and in other industries.

Proposed Course of Project:

This project is completed. See list of publications.

D. Isolation of fractions from common plants to be tested for their effect, either inhibitory or enhancing, or lack of effect, on tuberculin tests. When possible, the fractions will be tested for immunizing activity against tubercle bacilli.

Objectives:

To determine whether products from common plants (hemicelluloses, lipids or related fractions) have any relationship to tuberculin activity, or to the allergy or immunity produced by previous infection, or by these fractions in individuals without infection. There are large numbers of individuals unaccountably tuberculin-positive in our population.

Methods:

Fractionation procedures are adapted as necessary from well-known procedures, especially those for fractionation of tubercle bacilli. Guinea pigs or mice are tested for effect of these fractions on tuberculin reaction. Known high fat foods will be tested, peanuts as an example.

Major Findings:

On the assumption that chemical structure determines immunological activity, fractionation procedures like those applied to tubercle bacilli, have been used on some common plants; a few lipid fractions demonstrated antitubercular activity in vitro. Further work on this project awaits results of the chemical findings in respect to the current studies of several strains of tubercle bacilli.

Significance to Microbiological Research:

The long-distance aim of the project is to learn something as to the nature of the tuberculin test and its relation to immunity to tubercular infections. If fractions of common plants demonstrate any effect, then knowledge of immunity or lack of it may be somewhat clarified.

Proposed Course of Project:

Fractionation of various plants will be continued. Study of test conditions for both the tuberculin reaction and allergy will be made to adapt them for this project.

E. Development of procedures to determine whether immunological paralysis occurs against tubercular infections in laboratory animals similar to that found with pneumococcus polysaccharide.

Objectives:

To determine a method of measuring immunological paralysis and to test whether some fraction of the tubercle bacillus is responsible for the effect.

Methods:

Fractions are to be isolated and tested in varying concentrations to determine their ability to immunize or to produce immunological paralysis in mice. The effect on tuberculin reaction of various concentrations of such fractions may also be an indicator.

Major Findings:

A previous experiment indicated that intact microorganisms produce a state similar to immunological paralysis. Additional fractions have been isolated for testing the significance of this phenomena.

Significance to Microbiological Research:

If immunological paralysis is confirmed in tests of fractions of tubercle bacilli, then the animals so treated may be (similar to those in the pneumococcus study) hypersusceptible to infection.

Proposed Course of Project:

Further work on this project awaits results of the findings in the current cultural studies of several strains of tubercle bacilli.

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12. BUDGET ACTIVITY:

RESEARCH

13. None
IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATION PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957

14. None
IF THIS PROJECT RESEMBLES, COMPLEMENTS OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PHS (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS) IDENTIFY SUCH RESEARCH

Honors, Awards, and Publications Sheet

15.

16.
LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955.

FELTON, Lloyd D., Kauffmann, Gladys, Prescott, Benjamin, Ottinger, Barbara: Studies on the Mechanism of the Immunological Paralysis Induced in Mice by Pneumococcal Polysaccharides. J. Immunol., 74: 17-26, 1955.

FELTON, Lloyd D., Prescott, Benjamin, Kauffmann, Gladys, Ottinger, Barbara: Pneumococcal Antigenic Polysaccharide Substances from Animal Tissues. J. Immunol., 74: 205-213, 1955.

FELTON, Lloyd D., Prescott, Benjamin, Kauffmann, Gladys, Ottinger, Barbara: Antigens of Vegetable Origin Active in Pneumococcus Infections. J. Bact., 69: 519, 1955.

PRESCOTT, Benjamin, Kauffmann, Gladys, James, Walter D.: The "Pyridyl" Test for Hydrazine Assay in Blood Plasma. A.M.A. Arch. Indus. Health, 12: 393, 1955.

IN PRESS:

FELTON, Lloyd D., Prescott, Benjamin, Kauffmann, Gladys, Ottinger, Barbara: Pneumococcal Antigenic Polysaccharide Substances from Human Tissues. J. Immunology.

PRESCOTT, Benjamin, Kauffmann, Gladys, James, Walter D.: A Means of Reducing Hydrazine Toxicity in Mice. A.M.A. Arch. Indus. Health.

17. None
HONORS OR AWARDS FOR THE PROJECT

R. P. C. - 1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

1. National Microbiological Institute

2. Laboratory of Infectious Diseases
LABORATORY OR BRANCH

3. Bact. & Mycotic Diseases
SECTION

4. Bethesda, Md.
LOCATION

5. NMI - 36
SERIAL NO.

6. Chemical Analysis of Normal and Tuberculin Lung Tissue
PROJECT TITLE

7. J. Webb
PRINCIPAL INVESTIGATOR

8. Byron J. Olson
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

To Study Relationships Between Amounts of Silica, protein, deoxyribose nucleic acid (DNA), ribose nucleic acid (RNA) and total nucleic acids in histological sections of lung tissue (normal and pathological).

Objective:

Since the main objective was to study changes and differences in the tissue sections, it was necessary to have adequate methods of assay for the various constituents. For example, known methods of assay for DNA and RNA were not adequate because of lack of specificity and lack of sensitivity when only small amounts of tissue were available.

Accordingly, the first objective was to find more suitable reagents and analytical procedures for nucleic acid determination. An intermediate objective was to correlate these nucleic acid findings with results of other analyses performed on the tissue sections.

Methods:

A new method for DNA assay, based on the reaction of P-nitrophenylhydrazine with deoxyribose, and a new method for RNA assay, based on the reaction of P-bromophenylhydrazine with furfural from ribose, were used.

For other assays, conventional methods or modifications thereof have been employed.

Major Findings:

The new reagent for DNA assay (P-nitrophenylhydrazine) has proved to be more specific and has shown greater sensitivity than diphenylamine, the reagent usually employed.

The reagent used for RNA assay (P-bromophenylhydrazine) has demonstrated greater specificity and sensitivity. Preliminary experiments have been suggestive of its usefulness for determining RNA in formalin-fixed tissues. (Difficulties were encountered with this material when conventional methods were applied thereto).

Significance to Microbiological Research:

The development of these methods makes possible study of the relationship between nucleic acids and the extent of lung pathology and the study of differences where both right and left lungs are exposed to the same tuberculigenous agent.

Specific and sensitive methods for nucleic acid determination are of importance in other phases of microbiological research.

For example, studies concerned with metabolic processes of the cell; of tubercle bacilli or other microorganisms. Another example could be biochemical studies concerned with bacterial viruses.

Proposed Course of Project:

1 - Application of these nucleic acid methods to available pulmonary tissue,

2 - Further development of methods,

3 - Correlation of the results of the several analyses (silica, protein, dry weight, RNA, DNA and total nucleic acids).

Budget Data Sheet

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12. BUDGET ACTIVITY:

RESEARCH

13. None

IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATION PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957

14. None

IF THIS PROJECT RESEMBLES, COMPLEMENTS OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PHS (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS) IDENTIFY SUCH RESEARCH.

Analysis of NIH Program Activities

SERIAL NO. NMI -36

Honors, Awards, and Publications Sheet

15.

16.

LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955

"A sensitive method for the determination of deoxyribose nucleic acid in tissues and microorganisms". James M. Webb and Hilton B. Levy, Journal Biol. Chem. 213: 107 (1955).

"A sensitive method for the determination of ribose nucleic acid in tissues and microorganisms", Junius M. Webb. (Submitted to Journal Biol. Chemistry for publication)

17.

HONORS OR AWARDS FOR THE PROJECT

None

R. P. C. - 1　　Analysis of NIH Program Activities
December 1955

Project Description Sheet

1. National Microbiological Institute
INSTITUTE

2. Laboratory of Infectious Diseases
LABORATORY OR BRANCH

3. Bact. & Mycotic Diseases
SECTION

4. Bethesda, Md.
LOCATION

5. NMI - 37
SERIAL NO.

6. Chemical Studies of the Tubercle Bacillus
PROJECT TITLE

7. Dr. G. W. Lones
PRINCIPAL INVESTIGATOR

8. B. J. Olson, Dr. B. Prescott, Miss G. Kauffmann
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

A. Investigation of the Relationship between Metabolism and Isoniazid Resistance in the Tubercle Bacillus.

OBJECTIVE:

There have been a number of reports that strains of tubercle bacilli that have acquired resistance to the therapeutic agent isoniazid have also lost their catalase activity. It is the objective of this project to further examine the metabolism of resistant strains and to compare them with sensitive strains with the expectation of furthering our knowledge of the mechanism of resistance and sensitivity to isoniazid. Specifically, since catalase activity is dependent upon a ferriporphyrin-containing enzyme, the porphyrin relationships of sensitive and resistant cells will be studied.

Methods Employed:

Catalase determinations are made in the Warburg respirometer at low temperatures. Peroxidase determinations depend upon the development of a colored substance from benzidine and hydrogen peroxide. Resistant and sensitive strains of tubercle bacilli are grown and harvested, preferably on porphyrin-free media. The organisms are then examined for porphyrins by chemical treatment and solvent extractions followed by solvent fractionations, and finally, quantitative measurements with a photofluorometer. A spectrophotometer is necessary to obtain evidence for the presence or absence of the cytochromes in isoniazid-resistant strains. The cytochromes, like catalase and peroxidase, are porphyrin-containing substances.

Major Findings:

A procedure for obtaining reproducible measurements of catalase activity in the tubercle bacillus has been developed. The absence of catalase activity in three strains of isoniazid-resistant tubercle bacilli has been confirmed. It has also been found that the three strains possess no peroxidase activity. Both catalase and peroxidase were found in the parent sensitive strains from which these resistant strains were derived. Successful cultivation of these organisms on a porphyrin-free medium has now been accomplished. Examination for the members of the cytochrome series is in progress.

Significance to Microbiological Research:

The importance of an understanding of the mechanism by which a microorganism becomes resistant to a therapeutic agent is obvious. Added interest to this particular problem in Mycobacterium tuberculosis arises from the observation of others that isoniazid-resistant strains of the tubercle bacillus have a greatly reduced virulence, at least for the guinea pig. A better understanding of the altered metabolism of these strains may contribute to our knowledge of the factors influencing virulence and invasive ability.

Proposed Course of Project:

It is proposed next to examine the resistant strains for the presence of members of the cytochrome series known to be present in sensitive strains of tubercle bacilli. If these are absent, it would seem worth-while to determine the presence or absence of the porphyrins from which the cytochromes are derived.

B. Development of Chemically Defined Liquid Media for the Diffuse Growth of the Tubercle Bacillus.

Objective:

The objective of this project is to develop a chemically defined liquid medium supporting diffuse growth of the tubercle bacillus. Such a medium will permit the determination of growth rates by turbidimetric means for the study of nutritional requirements of the organism. Also, such a medium will be useful for the production of homogeneous populations of tubercle bacilli under defined nutritional conditions for metabolic studies.

Methods:

Since diffuse growth of the tubercle bacillus can be obtained in a Tween 80 - albumin medium, attempts have been made to modify the medium to permit the elimination of the albumin. Since albumin is reported to function as a detoxifying agent, attempts have been made to detoxify the Tween by other means.

Major Findings:

Previous indications of enhanced growth of the tubercle bacillus in the presence of alpha tocopherol and in the absence of Fraction V of bovine plasma albumin have not been confirmed by further study. Diffuse growth in the absence of added albumin equivalent to that in the presence of albumin has not been attained. However, a medium containing crystalline bovine plasma albumin, instead of the Fraction V bovine plasma albumin usually used, permits the growth of this organism in the absence of exogenous porphyrins found to be contained in Fraction V. This porphyrin-free medium is being used in the investigation of the relationship of isoniazid resistance to metabolism in the tubercle bacillus.

Significance to Microbiological Research:

The development of a chemically defined medium providing diffuse growth of the tubercle bacillus will contribute to research designed to add to our knowledge of the nutrition and metabolism of this organism. Moreover, the elimination of albumin should improve the reproducibility of culture, since this material is variable from lot to lot.

Proposed Course:

A detailed comparison of the growth promoting properties of Fraction V bovine plasma albumin and crystalline bovine plasma albumin appears to be necessary.

C. Investigation of the Metabolism of Nitrogen Compounds by Tubercle Bacilli.

Objectives:

It is the objective of this project to add to our knowledge of the nitrogen metabolism of this organisms by the application of some of the techniques which have proven fruitful for this purpose in other bacteria. The role of the metabolism of nitrogen compounds in problems of virulence and resistance is inadequately understood.

Methods:

Warburg respirometer and chemical analytical methods are used with such modifications as are dictated by considerations for the virulence of the organisms studied. A Warburg bath has been modified to provide for automatic pasteurization of the reaction vessels at the end of an experiment.

Major Findings:

The ability of resting cells of a BCG strain of the tubercle bacillus to deaminate 22 different amino acids has been examined. Little or no ammonia was produced from 11 of the amino acids tested. Slight but measurable ammonia production was obtained with 7 of the 22, and four were attacked at an appreciable rate. These are L-cysteine, L-serine, glycine, and L-asparagine. Of these, L-cysteine is also attached anaerobically. Hydrogen sulfide, ammonia and pyruric acid are products of the anaerobic reaction. It is of interest that L-cysteine and the related amino acid, L-cystine, are effective growth inhibitors for this organism. Conditions of growth and reaction influencing the deamination of several of the amino acids have been investigated.

Significance to Microbiological Research:

Our knowledge of the metabolism of the acid fast bacteria has lagged far behind that of many other microorganisms. It is hoped that improved knowledge of the metabolism of these organisms may contribute to a solution of some of the problems they create.

Proposed Course:

It is proposed to extend this study to other strains of the tubercle bacillus including virulent strains.

D. Isolation and Study of Cerolytic Enzymes. Initial phase will be isolation of Microorganisms capable of Producing Enzymes with the Ability to Hydrolyze the Waxes of the Tubercle Bacillus.

Objective:

A report from Egypt claims a remarkable therapeutic property in experimental tuberculosis for cerolytic extracts of certain wax digesting microorganisms. An attempt is being made to isolate such microorganisms. If successful isolations are made, it will be the objective of this project to purify the cerolytic enzymes, determine their properties and usefulness in tuberculosis research and treatment.

Methods:

Classical enrichment techniques in liquid media containing waxes, particularly tubercle bacillus wax, will be followed by isolation attempts on agar containing wax emulsions with and without suitable indicators. Possible inocula for the enrichment cultures include soil, sewage, digestive tracts of wax eating insects, etc.

Major Findings:

Little or nothing is known of the enzymes attacking waxes. Initial experiments on this project have been carried out with beeswax as a substrate. The use of enrichment cultures inoculated with soil followed by plating on solid media containing emulsified beeswax has resulted in the isolation of a Streptomyces sp. apparently capable of utilizing the wax, since it causes a clearing of the medium adjacent to the colony. This organism is being used as an aid in developing techniques for detection and estimation of wax hydrolysis. Preliminary tests suggest that it does not attack tubercle bacillus wax.

A considerable quantity of tubercle bacillus wax has been prepared. Difficulty has been experienced in obtaining dispersions of this material for use in growth media. Tubercle bacillus wax is quite different from beeswax in its physical properties. To date the best dispersions have been obtained by homogenizing, in a laboratory homogenizor, a solution of tubercle bacillus wax in pentane and an aqueous Tween 80 solution, followed by removal of the pentane in an autoclave.

Significance to Microbiological Research:

In addition to possible therapeutic activity of the "cerase" sought in this project, it is possible that such preparations might be useful research tools in the study of the chemical structure of the waxes of the tubercle bacillus and in the lysis of the tubercle bacillus to release intracellular enzymes for cell free metabolic studies.

Proposed Course of Project:

The enrichment and isolation rpocedure which was successful in isolating an organism capable of attacking beeswax will be applied to the search for organisms capable of degrading tubercle bacillus wax. If such isolations are successful, additional work will be dictated by the nature of the organisms and the enzymes involved.

Budget Data Sheet

10.

11.

12.

BUDGET ACTIVITY:

RESEARCH:

13. None

IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATION PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957

14. None

IF THIS PROJECT RESEMBLES, COMPLEMENTS OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PHS (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS) IDENTIFY SUCH RESEARCH.

Honors, Awards, and Publications Sheet

15.

16. None
LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

17. None
HONORS OR AWARDS FOR THE PROJECT

R. P. C. - 1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

1. National Microbiological Institute
INSTITUTE

2. Laboratory of Infectious Diseases
LABORATORY OR BRANCH

3. Bacterial and Mycotic Diseases
SECTION

4. Bethesda
LOCATION

5. NMI - 39
SERIAL NO.

6. The Effect of Animal Virus Reproduction on Host Cell Metabolism
PROJECT TITLE

7. Dr. Hilton Levy
PRINCIPAL INVESTIGATOR

8. Dr. Samuel Baron, D. B. S.
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives

To gain information relative to the relationships that exist between the diseased cell and the virus reproducing therein. It is anticipated that this program will elucidate some aspects of the mechanism of virus reproduction. In addition, better understanding of the nature of the disease response may be forthcoming from a determination of which the specific paths of cell metabolism are altered by the disease process. Knowledge of either or both of these phases of the problem (virus reproduction and altered cell metabolism) might be useful in the development of chemotherapeutic agents to inhibit virus growth and to aid the host.

Methods

The experimental approach to this program involves an attempt to infect uniformly a population of host cells with the virus under study, and then to examine by suitable laboratory techniques several metabolic systems. The cell-virus systems so far utilized are: (1) The Ehrlich Ascities tumor in Swiss mice, infected with Encephalomyocarditis virus, and (2) Monkey kidney tissue culture infected with polio virus. Uninfected tissue in each case is available as control material. The ascities tumor cells are desirable in that they provide large quantities of rather homogeneous biological material, but the difficulties of ensuring complete infection of the cell population, as well as the relatively small yield of virus for the amount of cells analyzed limit their usefulness. The tissue culture, on the other hand, gives a high yield of virus per weight of cells, and can be uniformly infected. The problems involved in getting large quantities of such cells, however, complicate certain types of analysis. To study the different phases of metabolism, different biochemical and biophysical techniques have been and will be employed. For the study of energy production and utilization, for example, combined manometry, chemical analysis, and energy rich phosphate bond turnover studies will be employed. Study of the effects of virus on protein composition will involve chromatographic amino acid analysis. Turnover of the amino acids, as well as possible interconversions of amino acids, will be followed largely through the use of radioactive carbon, etc.

Major Findings

A. Encephalomyocarditis virus in Ehrlich Ascities Tumor.

A paper has been submitted to the Journal of Infectious Diseases incorporating the following results.

(1) The purine and pyrimidine compositions of the desoxyribosenucleic acid and ribosenucleic acid isolated from a tumor cell population that has supported E.M.C. growth does not differ from those of control cells.

(2) The concentrations of acid soluble phosphorus, phospholipid phosphorus, ribosenucleic acid and desoxyribosenucleic acid from infected cells does not differ from the corresponding concentrations in control cells.

(3) The turnover rates of desoxyribosenucleic acid phosphorus and ribosenucleic acid phosphorus is significantly higher in infected cells than those of control cells, while the turnover rates of acid soluble phosphorus and phospholipid phosphorus is essentially the same in infected and control cells.

B. Poliomyelitis virus in monkey kidney cells.
(Done in cooperation with Dr. Samuel Baron, D.B.S.)

A paper is in preparation which will state the following:

Monkey kidney tissue culture cells were infected with Type III polio virus with a ratio of infectious doses of virus added/cells of approximately 40 so as to ensure infectivity of almost every cell. These results were obtained:

(1) Aerobic glycolysis of the infected cells within an hour after infection exceeds that of uninfected cells, and continues higher until shortly before the cells start to come off the glass. At this point the glycolysis of control cells becomes greater than that of the infected.

(2) The aerobic glycolysis of monkey kidney tissue culture is remarkably high. Ordinary monkey kidney slices show little or no glycolysis, but the tissue culture has a $Q^{O_2}_{CO_2}$ of 18-36, a figure generally reported only for tumor tissue.

(3) The uptake of radioactive glycine by infected cells is inhibited by type III polio virus within an hour after infection, and this inhibition continues until the death of the cells. Under the experimental conditions used, the uptake of glycine into protein was the major factor measured, and we cannot say what were the relative uptakes into other biochemical fractions.

Significance to Microbiological Research

The intensive investigations of viruses conducted at N.M.I. are devoted largely to epidemiological, clinical or immunological aspects of viral diseases. The program described here should contribute somewhat toward rounding out the biochemical and metabolic phases of the research. In addition, it is to be hoped that this program will be of use to, and will draw on the knowledge gained in the more clinically directed programs.

Proposed Course of Project

During the coming year there is planned:

(1) An investigation of possible enzymatic and/or permeability changes in the infected cells to seek an explanation of the rise in glycolysis.

(2) A study of the effects of the other polio types, as well as other viruses, to determine whether the above mentioned findings are universal or specific.

(3) If time permits an investigation of the effects of viruses on nucleic acid composition and metabolism in tissue culture.

Budget Data Sheet

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12. ______________________________

BUDGET ACTIVITY:

RESEARCH

13. ______________________________

IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATION PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957

Dr. Samuel Baron, Division of Biologics Standards

14. ______________________________

IF THIS PROJECT RESEMBLES, COMPLEMENTS OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PHS (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES, OR FUNDS) IDENTIFY SUCH RESEARCH.

NONE

Honors, Awards, and Publications Sheet

15.

16.

LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

Levy, Hilton B. and Snellbaker, LeRoy F.: Effects of Animal Viruses on Host Cell Metabolism. 1. Effects of E.M.C. virus on Nucleic Acids and Phosphorus Fractions of Ehrlich Ascites Tumor Cells. Accepted for publication. To appear in The Journal of Infectious Diseases

17.

HONORS OR AWARDS FOR THE PROJECT

None

R. P. C. - 1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

1. National Microbiological Institute
INSTITUTE

2. Laboratory of Infectious Diseases
LABORATORY OR BRANCH

3. Bacterial and Mycotic Diseases
SECTION

4. Bethesda
LOCATION

5. NMI - 40
SERIAL NO.

6. Nucleoproteins and Nucleic Acids as Antigens
PROJECT TITLE

7. Dr. Hilton Levy
PRINCIPAL INVESTIGATOR

8. Dr. Samuel Baron, D.B.S.
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives

In view of the biochemical specificity and genetically fundamental character of the nucleic acids, particularly desoxyribosenucleic acid, together with their large molecular size, it is planned to investigate their possible activity as antigens. The production of different types of antibodies will be studied, as well as possible immunizing action. Genetic similarities among different strains of organisms will be investigated by means of cross-reactivities of sera with non-homologous nucleic acids. The purine and pyrimidine composition of the ribosenucleic acids and desoxyribosenucleic acids isolated from the different strains of bacteria will be determined and the results analyzed in light of possible immunological similarities or relationships.

The program is restricted, at least for the present, to various strains of acid-fast bacteria.

Methods

We have developed new methods for the preparation of highly polymerized nucleic acids and nucleoproteins from a variety of bacteria. For this study we have prepared nucleic acids from the BCG, the avian and the Vole varieties of the tubercle bacillus. When suitable safety precautions have been worked out, preparation of nucleic acids from the human tubercle bacillus will be undertaken. Standard techniques will then be used for testing for antigenicity of the nucleic acids and for the development of immunity in the test animals. Paper chromatography will be used for the chemical analysis of the nucleic acids.

Major Findings

The ability of highly polymerized nucleic acids and nucleoproteins from *Mycobacterium tuberculosis* to act as antigen has been established. Three different strains of *Mycobacterium* were used. Varieties *bovis*-Strain BCG, variety *avium*, and the organism from the vole. Relatively crude mixtures of desoxyribonucleoprotein and ribosenucleoprotein, as well as deproteinized mixtures of deoxyribonucleic acid and ribonucleic acid were used to develop C. F. antibodies in rabbits and in mice. In the latter animal, at least, the administration of the antigen intraperitoneally was as effective as intravenous administration. Moderately high titers were obtained which persisted for at least 4-6 weeks and a number of cross reactions among the three varieties were found. Antigenic polysaccharides prepared from the same organisms gave no reaction to the nucleic acid antisera, indicating the probable absence of polysaccharides from the nucleic acid preparation. Dr. B. Prescott has prepared about 8 grams of nucleoprotein for future use.

Significance to Microbiological Research

The fact that antigenic activity has been demonstrated in nucleic acid preparations immediately raises the question as to the possible role of such antigens in immunological specificity. In view of the history of the poor relationship between production of circulating antibody and immunity in tuberculosis, it is not likely that this antigen will be of value in the prophylaxis of tuberculosis, but this possibility must be investigated. Whether nucleic acid antigens might prove useful in differential diagnosis of bacterial diseases could be investigated.

Proposed Course of Project

In cooperation with Dr. Olson and Dr. Prescott, we plan to determine whether or not these antigens produce any degree of immunity in guinea pigs. We also intend to determine which of the nucleic acids, ribonucleic acid or deoxyribonucleic acid, or both, is responsible for the observed antigenicity. If possible, nucleic acids from other organisms will be prepared and tested for antigenicity and cross reactions. We hope to analyze the various nucleic acids for purines and pyrimidines to see if there is any correlation between gross chemical make-up and cross-reactivity.

Budget Data Sheet

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12. BUDGET ACTIVITY:

RESEARCH

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATION PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957

Dr. Samuel Baron, Division of Biologics Standards

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PHS (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS) IDENTIFY SUCH RESEARCH.

None

Honors, Awards, and Publications Sheet

15.

16.

LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

Levy, Hilton B. and Snellbaker, LeRoy F.: On a Fluoresant Component of Deoxyribonucleic Acid Hydrolyzates. Arch. Biochem. Biophys. 54: 549-555, 1955.

Webb, Junius M. and Levy, H. B.: A Sensitive Method for the Determination of Deoxyribonucleic Acid in Tissues and Microorganisms. J. Biol. Chem. 213: 107-117, 1955.

17.

HONORS OR AWARDS FOR THE PROJECT.

None

R. P. C. - 1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

1. National Microbiological Institute
INSTITUTE

2. Laboratory of Infectious Diseases
LABORATORY OR BRANCH

3. Bacterial and Mycotic Diseases
SECTION

4. Bethesda
LOCATION

5. NMI - 41
SERIAL NO.

6. Iron Metabolism in Infections and in Hypersensitive Disease States
PROJECT TITLE

7. A. L. Schade
PRINCIPAL INVESTIGATOR

8. Jiro Oyama
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

A. Methods for the determination of the total iron-binding capacity of blood plasma, the amount of bound iron in plasma, and the amounts of free and bound iron in microbiological media.

Objectives

To perfect methods for the determination of iron, both free and bound, in body fluids and in media of microbiological importance to aid in the study of the iron metabolism of the host as well as of the infectious agents. Attention is directed especially to accurate iron estimations in small amounts of test fluids, ca. 0.1 ml, and to minute amounts of iron, ca. 0.05 ug.

Major Findings

Iron analyses can be quickly and accurately performed on as little as 0.1 ml of plasma with a reproducibility of ± 5%. No protein removal is required either in the estimation of siderophilin-bound iron or of the total binding capacity of the plasma sample. The methods as outlined in the Proc. Soc. Biol. & Med. 87:443-448, 1954 have been extended and refined to facilitate the determination of such micro amounts. There is evidence at hand of world wide interest and adoption of the proposed methods.

Significance to Microbiological Research

The availability of a simple, reliable method for the total iron-binding capacity of blood is of value for the study of all disease states in which iron metabolism is disturbed by infectious disease or otherwise. In particular, current studies on the iron metabolism of patients suffering from Agammaglobulinemia are in progress in cooperation with Dr. C. Martin of L.C.I. to determine the significance of the amount of bound iron and the total iron binding capacity of the plasmas from these cases. Cases of Coxsackie virus infection, coccidiomycosis, sarcoidosis, etc., have been analyzed in cooperation with Drs. Kushner, Louria, and Merchant of L.C.I. In addition, studies of plasma iron metabolism in sprue and in leukemias have been and are being pursued in collaboration with Dr. J. Stengle, now of N.C.I.

The developed analytical methods are of value in the study of the particular iron requirements of microbes (protozoa, bacteria, and viruses) not only quantitatively but also qualitatively. Toxin production by such bacteria as the Corynebacterium and Shigella may more readily be investigated through use of those methods.

Proposed Course of Project

We plan to continue the application of our methods to plasma from various disease states and to refine them, if necessary, to increase their general usefulness.

B. Ultraviolet Irradiation of Blood Bank Plasma and Its Effect on the Iron-Binding Protein, Siderophilin.

Objectives

To determine the effect of U.V.R. on blood plasma in terms of a specific protein component and to relate such effect quantitatively to the amount of radiation given.

Methods

In addition to the use of the uranyl oxalate actinometric method for the quantization of radiation, we employed our published methods for the determination of bound plasma iron and the unsaturated iron-binding capacity of the plasma sample.

Major Findings

The results show that the U.V.R. siderophilin inactivation initially follows an apparent first order reaction, then departs from the first order to disintegrate into a complex decomposition by reason, in part, of the production of highly UV-absorbent end products. Further, the loss in iron-binding capacity of the plasma is due to the UV inactivation of both the iron-complexed and the iron-free siderophilin. Both forms of siderophilin are inactivated at the same rate.

Significance to Microbiological Research

Since it is our position that the function of siderophilin is not only to carry the iron employed in hemoglobin formation to the erythropoetic tissues but also to function as an in vivo bacteriostatic agent through iron deprivation for possible infectious organisms, it is of importance to know the sensitivity of this protein to ultraviolet radiation per se and of blood bank plasma to be administered to human recipients.

Proposed Course of Project

The project is completed and the results ready for publication.

C. Serum Iron, the total Iron-binding Capacity of Blood, and Immunity to Infection by Bacteria, Viruses, and other Infectious Agents.

Objectives

The aims of this project are to investigate the relationships of the amount of iron bound to siderophilin in blood serum as well as the concentration of iron-free siderophilin to the phonomenon of infection, to immunity therefrom, and to study the possible therapeutic implications of such relationships.

Methods

The usual immunological, biochemical, radioactivity, and bacteriological procedures are employed. In addition, methods peculiar to both conalbumin and siderophilin affinities for iron serve.

Major Findings

Investigations have shown that pathogenic bacteria, as well as saprophytic forms in a generally satisfactory growth medium, can be divided into three groups on the basis of their growth reaction to the addition of siderophilin or conalbumin to the medium. They are either completely, partially, or not at all inhibited by the siderophilin addition. Both the complete and partial inhibition is overcome by addition of an amount of iron in excess of the binding capacity of the added specific iron-binding protein. Human and rabbit blood sera which possess unsaturated iron-binding capacity occasion similar effects in addition to other inhibitory properties against certain bacterial forms.

To establish "normal" values of both bound plasma iron and total iron-binding capacities we, in collaboration with Dr. James Stengle of L.C.I., analyzed the plasma iron and iron-binding capacity levels of 12 male and 10 female "normal" volunteers over a 24 hour period by obtaining 8 blood samples per donor at roughly 3 hour intervals.

Correlations of hemoglobin, hematocrit, white blood cell count, copper levels, bilirubin, total serum protein, serum albumin, and 2 glycolytic enzyme levels with the iron values of all 176 blood samples were made. The results obtained are being readied for publication. An unexpected finding was the statistically significant difference in total serum protein levels of men as compared to women; the men having a higher concentration of serum protein than the women.

Significance to Microbiological Research

The serum or plasma iron levels, as well as the concentration of siderophilin in the blood, are valuable indicators of infectious disease states. The serum iron picture reflects the iron metabolism of the host and provides a useful tool for investigation of anemic conditions generally associated with infections. Further, knowledge of the metabolism of iron, particularly by the leucocytes and the reticulo endothelium system as reflected by the serum iron levels is probably of significant value for the mobilization and activity of these agents in hypersensitivity reactions and immunological defense mechanisms of the host.

Proposed Course of Project

Studies on the iron requirements of pathogenic and saprophytic bacteria with emphasis upon the available sources of iron for those organisms capable of growth in human serum despite its excess iron-free siderophilin; the effects of siderophilin on the metabolism of those bacteria requiring inorganic iron for growth; the mechanism by which the host removes iron from the iron siderophilin complex and maintains the ratio of per cent iron saturated and iron-free siderophilin in blood plasma; the serum iron picture as it appears in the various infectious disease conditions under observation and study in L.C.I. and, particularly, an unusual serum iron and total iron-binding capacity situation that has been under investigation in collaboration with Dr. Christopher Martin of L.C.I. in cases of Agammaglobulinemia will be pursued.

D. Metabolism of Leucocytes as Related to Hypersensitive States, Host Reaction to Infection, and Leukemia.

Objectives

To investigate the respiratory activity and iron metabolism of leucocytes in normal, hypersensitive and leukemic states.

Methods

1. Isolation of leucocytes from red cells by rouleaux, precipitation; 2. Warburg respiration procedures; 3. radioactivity and chemical iron determinations; 4. appropriate immunological and microscopical procedures.

Major Findings

Preliminary study has resulted in the development of a rapid, satisfactory procedure for the isolation of leucocytes from blood.

Significance to Microbiological Research

The intimate metabolism of the cells responsible for hypersensitivity and immunity is poorly understood and deserving of much investigation.

Proposed Course of Project

Initial studies will be done on leucocytes of normal and leukemic subjects in collaboration with Dr. James Stengle of N.C.I.

R. P. C. - 2 Analysis of NIH Program Activities SERIAL NO. NMI - 41
December 1955

Budget Data Sheet

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12. BUDGET ACTIVITY

Research

13.

IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATION PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957

Dr. James Stengel, National Cancer Institute

14.

IF THIS PROJECT RESEMBLES, COMPLEMENTS OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PHS (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS) IDENTIFY SUCH RESEARCH.

None

Honors, Awards, and Publications Sheet

15.

16.

LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

Schade, Arthur L.: Plasma Iron: Its Transport and Significance. Nutrition Reviews 13:225-227. 1955. (Lead article written on request of the Nutrition Foundation per Dr. Frederick Stare.

Schade, Arthur L. and Oyama, J.: Effect of Ultraviolet Radiation on Iron-binding Capacity of Plasma. Accepted for publication. To appear in the Proc. Soc. Biol. and Med.

R. P. C. - 1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

1. National Microbiological Institute
INSTITUTE

2. Laboratory of Infectious Diseases
LABORATORY OR BRANCH

3. Bacterial and Mycotic Diseases
SECTION UNIT

4. Bethesda
LOCATION

5. NMI- 42
SERIAL NUMBER

6. Brucellosis
PROJECT TITLE

7. Dr. Norman B. McCullough
PRINCIPAL INVESTIGATOR

8. Dr. Grace A. Beal
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

A. Brucellosis

Objectives:

The objects of this project are broad and studies are conducted on all aspects of brucellosis, including the nutrition, metabolism, and enzymatic make-up of the organism, mechanism of action of antibiotics on the organism, factors determining virulence of the organisms, the development and efficacy of prophylactic immunizing agents, field studies to determine the occupational hazard of the disease, epidemiology, diagnosis and therapy of the disease in man.

Methods Employed:

The Warburg technique is employed in enzymatic and metabolic studies; growth in a synthetic medium is used for nutrition experiments; guinea pig inoculation for virulence and vaccine studies; occupationally exposed groups for epidemiological studies; human patients for studies on diagnosis and therapy.

Major Findings:

A study pursued over the last several years on the correlation of colony type with virulence of the organisms for the guinea pig has been completed and the data are being prepared for publication. For all three species of Brucella, organisms from S type colonies have proved infectious for the guinea pig when very small numbers were inoculated. There is a strict correlation between S type colony and guinea pig virulence. Colony types showing only minor detectible differences from the S type

Major Findings (continued):

have greatly reduced virulence for the guinea pig.

S type cells in guinea pig spleens (intracellular) remain viable and show no loss in virulence for the guinea pig when stored in the deep freeze for a period of 3 years. Organisms suspended in a variety of milieu and similarly stored relatively quickly lose viability and virulence. Loss in virulence is associated with change in colony type. This material is being prepared for publication.

A study on streptomycin dependent cultures pursued over the last four years has been completed. A stable dependent culture, properly manipulated reverted to a non-dependent status after 3-1/2 years. The recovered strain had a virulence for the guinea pig identical to that of the parent culture and the colony type likewise was the same. This data argues against the mutation theory of origin of dependent cultures. This material is being readied for publication.

A collaborative study with the Veterinary Department of Purdue University is underway on the effect of residence in swine on certain characteristics of old-world melitensis strains. This study is an effort to clarify the systematic position of so-called American type melitensis cultures.

Significance to Microbiological Research:

From a public health standpoint, in the United States, brucellosis is the most important disease of animals transmissible to man, is the cause of serious economic loss to the livestock industry, is an occupational disease, and is one which presents many problems in the diagnosis and therapy of the infection in man. The organism readily lends itself to basic studies outlined elsewhere in this report.

Proposed Course of Project:

Continue as outlined above. In addition major emphasis will be directed toward a study of the relative roles of circulating antibody and tissue immunity (hypersensitivity) in determining resistance of guinea pigs to infection with highly virulent cultures administered by various routes. This is a fundamental study utilizing a unique phenomenon for production of immunity with and without accompanying allergy.

Budget Data Sheet

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12. BUDGET ACTIVITY:

Research

13. IDENTIFY ANY COOPERATIVE UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATION PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957.

Animal Disease Station and Personnel Branch, U. S. Department of Agriculture.
Veterinary Department, Purdue University.

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PHS (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS) IDENTIFY SUCH RESEARCH.

This project is supplemented by the brucellosis studies done on patients in the Laboratory of Clinical Investigations, National Microbiological Institute, under the direction of the same Principal Investigator.

Honors, Awards, and Publications Sheet

15.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

None

17. HONORS OR AWARDS FOR THE PROJECT

None

R. P. C. - 1 Analysis of NIH Program Activities

Project Description Sheet

1. National Microbiological Institute
INSTITUTE

2. Laboratory of Infectious Diseases
LABORATORY OR BRANCH

3. Basic Studies
SECTION

4. Bethesda
LOCATION

5. NMI - 43
SERIAL NO.

6. Investigations on poliomyelitis
PROJECT TITLE

7. Dr. Karl Habel
PRINCIPAL INVESTIGATOR

8. Dr. Alexis Shelokov and Dr. John W. Hornibrook
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

A. Epidemiological investigations of poliomyelitis.

Objectives:

Determine the reservoir, mode of spread and factors responsible for epidemic and endemic occurrence of poliomyelitis.

Methods:

Stool and blood specimens are collected from various population groups representing pertinent epidemiological situations in respect to poliomyelitis. Using tissue culture technics in the laboratory these specimens are processed for the presence of virus which is subsequently typed immunologically and for the presence of specific poliomyelitis antibodies. Correlations are then sought between clinical and epidemiological findings in the field and the specific etiological results in the laboratory.

Major Findings:

Acting as a collaborating laboratory in the U. S. Public Health Service Polio Surveillance Program for Maryland, Virginia and the District of Columbia, approximately 1400 samples of material from cases suspected of being poliomyelitis were processed in our laboratory from May to August 1955. For most of the patients epidemiological and clinical data were not made available to us so this represented a "service" type of operation which halted most all research in this laboratory for 3 months. However in the early part of this work poliovirus was isolated from members of families of children who had recently been vaccinated with the Salk vaccine.

A specific study of 400 children for the presence of poliovirus was carried out on 200 younger siblings of vaccinated children and 200 similar individuals in families of unvaccinated children of the same age group. Completed in June this study showed no indication of secondary polio infection in younger siblings of children who 3 to 5 weeks previously had received Salk vaccine (Wyeth).

Significance to Microbiological Research:

Most of the activity in this project was due to difficulties arising in the polio vaccine program. Our activity was significant insofar as it helped elucidate some of the practical problems involved in the use of the Salk vaccine in 1955.

Proposed Course of the Project:

No further work in the area of this year's activity is planned. Antibody studies on sera from nurses working on polio wards planned for this year, but interrupted by our service operation, will be completed.

B. Evaluation of the clinical types of poliomyelitis from etiological point of view:

Objectives:

To determine if polioviruses are really the cause of all clinical disease diagnosed as poliomyelitis and to investigate factors which determine the severity of the clinical disease.

Methods:

Specific etiological studies by virus isolation or antibody demonstration are applied to specimens from large numbers of paralytic and nonparalytic or minor illness cases occurring in the summer months. Further laboratory tests are carried out in experimental polio to determine physiological factors that may influence the occurrence of severe paralysis in this disease.

Major Findings:

Major interest has been in patients suffering from a clinical disease frequently diagnosed as "nonparalytic polio" or "aseptic meningitis". Besides typable polioviruses, Coxsackie B viruses of Types 1, 2, 3, 4 and 5 have been isolated from groups of these patients along with several as yet unidentified viruses.

A very tedious and time-consuming job has been concerned with attempts at immunological typing of large numbers of newly isolated nonpolio viruses. This has been done by preparation of 15 different antisera and quantitative cross neutralization of each of the 15 viruses against all the antisera. Although not yet complete there is already evidence of subgrouping among the B Coxsackie viruses not previously recognized.

Furthermore, the greater efficiency of monkey kidney tissue culture over suckling mouse inoculation for isolation of B Coxsackie viruses is apparent.

Significance to Microbiological Research:

The identification, classification and characterization of new viral agents isolated from human beings is necessary before their significance in human disease can be scientifically investigated. Already it is obvious that Coxsackie B viruses, not poliovirus, is a frequent cause of "nonparalytic polio".

Proposed Course of Project:

Further efforts to classify and characterize the agents already isolated will be followed by serological surveys accompanied by epidemiological and clinical investigations to determine what human illnesses may be caused by these new viruses.

C. Basic Studies on Virus-Host Cell Relationships

Objectives:

To learn more information on how a virus invades a cell, multiplies within it and is released.

Methods:

Measurement of the susceptibility of various types of human and animal cells in tissue culture to multiplication and cytopathogenic effects of a variety of viruses. (In collaboration with Dr. Earle of NCI, Dr. Eagle, NMI, and Dr. McBride of NIDR.) Direct observation of effects of virus infection on cells using special "in vivo" tissue culture chamber technic and phase microscopy. (We supply tissue culture and do virus aspects in collaboration with Dr. DuBuy, LID, NMI.) Through the use of synthetic media for HeLa cells in tissue culture the nutritional requirements of cells for the support of viral multiplication are being determined. (We do viral aspects in collaboration with Dr. Eagle, NMI.)

Major Findings:

After Dr. Earle had adapted HeLa cells to growth in horse serum media rather than human serum, we demonstrated the cells to still be equally susceptible to polio in the practical uses made of these cells for poliovirus assay and isolation.

The susceptibility of normal skin cells (Dr. Earle) and a new human tumor epithelial cell (Dr. Eagle) to a spectrum of viruses has been completed. Several viruses such as yellow fever, lymphocytic choriomeningitis and mumps viruses have been successfully grown in these tissues whereas they are not easily propagated in other cell types.

Glucose and glutamine have been shown to be essential materials in the tissue culture medium for the HeLa cell to support growth of poliovirus.

Chronic latent infection of HeLa cells with Type III poliovirus has been maintained for 5 months.

Proposed Course of Project:

Other virus-cell systems will be investigated for the effect of glucose and glutamine on viral growth with an attempt to pinpoint the exact mechanism of this effect. Effect of medium constituents in determining the development of cytopathogenic effect of certain viruses will be studied.

Using the special tissue culture chamber the viral events will be correlated with cellular events for cytopathogenic and non-cytopathogenic viruses.

R. P. C. - 2 Analysis of NIH Program Activities SERIAL NO. NMI -43
December 1955

Budget Data Sheet

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12. BUDGET ACTIVITY:

RESEARCH

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATION PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957

On Project 3: Dr. Eagle's Section on Experimental Therapeutics, LID
Dr. Earle's Tissue Culture Section, Nat. Cancer Inst.
Dr. McBride, Nat. Inst. of Dental Research

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PHS (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS) IDENTIFY SUCH RESEARCH.

Project 1: Resembles some work done at CDC, Montgomery, Ala.
Projects 1 & 3: Resembles some work done at Rocky Mountain Laboratory, Hamilton, Montana
Project 3: Resembles some work done by Division of Biologics Standards.

R. P. C. - 3 Analysis of NIH Program Activities SERIAL NO. NMI - 43
December 1955

Honors, Awards, and Publications Sheet

15.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

"Relation of Poliomyelitis Virus Types to Clinical Disease and Geographic Distribution: A Preliminary Report," by Alexis Shelokov, Karl Habel, and Donald W. McKinstry; published in the Annals of the N. Y. Acad. of Sci., Vol. 61, Art. 4, pp. 998-1004, Sept. 27, 1955.

"Susceptibility to Poliomyelitis Virus of HeLa Cells Adapted to Growth in Horse Serum Medium", by Karl Habel, Nancy C. Gregg, and W. D. McBride, published in Proc. Soc. Expl. Biol. & Med., 90:87-89, Oct.1955.

"Laboratory Data Supporting the Clinical Trials of Antirabies Serum in Persons Bitten by a Rabid Wolf., " by Karl Habel and Hilary Koprowski, published in Bull. Wld. Hlth. Org., vol. 13, No. 5, 773, 1955.

"Relative Pathogenicity of Newly Isolated Strains of Poliomyelitis for Monkey Kidney Cells and Mice," by Rosalie J. Silverberg, Karl Habel and Alexis Shelokov, accepted by Proc. Soc. Expl. Biol. & Med.

"Subclinical Poliomyelitis in a Newborn Infant due to Intrauterine Infection," by Alexis Shelokov and Karl Habel, accepted by J.A.M.A.

17. HONORS OR AWARDS FOR THE PROJECT

None

R. P. C. - 1 Analysis of NIH Program Activities
December 1955

Project Description Sheet

1. National Microbiological Institute
INSTITUTE

2. Laboratory of Infectious Diseases
LABORATORY OR BRANCH

3. Basic Studies
SECTION

4. Bethesda
LOCATION

5. NMI - 44
SERIAL NO.

6. The Biology of Mitochondria and its Relation to Endogenous and Viral Diseases
PROJECT TITLE

7. H. G. DuBuy
PRINCIPAL INVESTIGATOR

8. Carl F. T. Mattern, Marie L. Hesselbach and Jane L. Showacre
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

A. Mitochondrial differentiation (H. duBuy)

Objectives

Morphological and chemical comparison of normal, modified and mutated mitochondria.

Methods

Methods encompass application of cytological, cytochemical, chemical and metabolic techniques on whole cells and isolated subcellular elements, in order to establish basic patterns common and to demonstrate differences due to changes in the mitochondria. Various media are used to extract subcellular elements in as nearly unchanged conditions as possible. This is determined by staining and biochemical techniques.

Major Findings

A number of media have been worked out from which sucrose was eliminated allowing phosphorylation determinations.

Significance to Microbiological Research

Differences between normal mutated and virus infected mitochondria can be established experimentally.

Proposed Course of Project

Continuation.

B. Cultivation of mitochondria in vitro (H. duBuy)

Objectives

Cultivation of mitochondria in vitro.

Methods

Using the methods of project A, in addition to information obtained from the subsequent projects, the maintenance of form and function of isolated mitochondria is being studied over increasing time periods.

Major Findings

Mitochondria could be kept enzymically active for at least 8 hours at 38°C.

Significance to Microbiological Research

On the basis of further elucidation of the nature of normal and abnormal subcellular particulates, an empirical approach to medical treatment can eventually be supplanted by a rational approach.

Proposed Course of Project

Further substantiation of the theory presented thus far by further data obtained by the projects presented.

C. Localization of enzyme systems on mitochondria (M.L. Hesselbach)

Objectives

Localization on the mitochondria of the major enzyme systems of the cell.

Methods

Methods encompass biochemical and metabolic techniques on subcellular elements. In the past year major efforts have been directed towards demonstration of the carbohydrate enzymes of the cell on the mitochondria. This phase has been finished. The project will be further pursued with regard to amino acid metabolism.

Major Findings

Localization of a number of carbohydrate metabolizing enzyme systems on brain and liver mitochondria.

Significance to Microbiological Research

Study of the complete biochemical potentialities of normal mitochondria will allow comparison with experimentally altered mitochondria.

Proposed Course of Project

Shifting of major effort to amino acid metabolism.

D. Carcinogenesis and mitochondria (M.L. Hesselbach)

Objectives

Relation of mitochondria to carcinogenic agents.

Methods

Methods encompass the study of the localization of the action of certain carcinogens in the cell, using the biochemical interrelation of the mitochondria and the other cell fractions with the chemicals.

Major Findings

None

Significance to Microbiological Research

Establishment of the intracellular site of action of chemicals which are known to change cellular metabolism.

Proposed Course of the Project

Elaboration of the project.

E. Subcellular virology (C.F.T. Mattern)

Objectives

(1) To determine the role of subcellular particles in virus multiplication and antibody formation; (2) To purify virus preparations and to study them with respect to chemical composition, antibody formation, and antigen-antibody reaction: also in respect to observed crystal instability; (3) To develop improved methods of counting small particles (less than 1 micron) to permit better quantitation in subcellular work.

Methods

(1) Methods described under other projects have been used for preparation of subcellular fraction; (2) Methods of purification were adapted from various procedures of protein purification; (3) Method for counting particles described in an article presented for publication.

Major Findings

(1) Preliminary results only. (2) A Coxsackie A virus has been purified and concentrated to such an extent that two different crystalline forms, of high infectious titer, have been obtained, under different conditions. These crystals are quite unstable rendering detailed study difficult. (3) A detailed study of electrical "gating" as a method of counting particles of sizes 3 to 9 microns and determining their size distribution has been completed. These results have been prepared in manuscript form and submitted for publication. Because of the promise of the method studies of particle counting in bacterial and subcellular particle size ranges are being undertaken.

Significance to Microbiological Research

These studies are all aimed at a better fundamental understanding of the nature of virus multiplication by using purified viruses, isolated, fractionated, subcellular particles and quantitative methods. The method of particle counting offers an extremely useful tool for definition of microscopic particles.

Proposed Course of Project

To continue as described above, and develop adequate techniques for studying the role of isolated subcellular fractions in virus multiplication and antibody production.

In addition to primary research duties, one of the members of this Unit has been assigned, since June 1955, part time (25%) to the Division of Biologics Standards. This has involved six "inspection" trips to Eli Lilly and Company's poliomyelitis vaccine production facilities, attending numerous meetings of and reporting to the Poliomyelitis Vaccine Technical Committee, and analyses of Lilly vaccine protocols for specific information. Two research projects have been undertaken in conjunction with the Polio Vaccine: one involving counting of particulate debris in vaccine, the other related to explaining other anomalies in inactivation of the virus, and is being undertaken with Dr. Samuel Baren, D.B.S.

F. Characterization of mitochondria (J.L. Showacre)

Objectives

To discover the biochemical nature of the specificity of mitochondrial subvital staining with Janus green B.

Methods

The staining, destaining and restaining of mitochondrial elements, following the application of subvital stains, is studied with various microscopic techniques. Biochemical reactions are studied with the Warburg technique. This project has been expanded to include other dyes, and has been finished. The information gained has established new reliable criteria for the definition of intact mitochondrial elements. It has also supplied a useful tool for the demonstration of the presence of certain dehydrogenases in various preparations resulting from cell fractionation. This information is being applied to project E.

Major Findings

Analysis of the mitochondrial staining reaction with Janus green B.. Use of Janus green B and Phenazine to distinguish between glycolytic and Krebs cycle dehydrogenases. (One article published, one in preparation.)

Significance to Microbiological Research

Establishment of a method to properly identify mitochondria.

Proposed Course of Project

Nearly completed.

G. Cytopathogenic effect in single cells in tissue culture (J.L.Showacre)

Objectives

To study the effects of virus infection on single cells grown in tissue culture.

Methods

Single tissue culture cells are studied microscopically in phase contrast and dark field, using subvital staining reactions to determine changes in dehydrogenase activity, and the localization of these changes on the cell. Micro-photographic records are made.

In this manner initial cellular reactions to the virus can be elucidated.

Major Findings

None

Significance to Microbiological Research

A more detailed study of the cytopathogenic effect might lead to a differentiation of the action of different viruses.

Proposed Course of Project

Continuation.

Budget Data Sheet

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12. BUDGET ACTIVITY:

RESEARCH

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATION PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 OR 1957

Chemistry Section, NIAMD
Division of Biologics Standards

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PHS (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS) IDENTIFY SUCH RESEARCH.

None

Honors, Awards, and Publications Sheet

15.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

On the Enzymic Nature of Mitochondrial Characterization by Janus green B and the Detection of Krebs-cycle Dehydrogenases with Janus green B. Jane L. Showacre and H. G. duBuy, Journal of the National Cancer Institute, Vol. 16, No. 1, August 1955.

17. HONORS OR AWARDS FOR THE PROJECT

None

R. P. C. - 1 Analysis of NIH Program Activities
December 1955

Project Description Sheet

1. National Microbiological Institute
INSTITUTE

2. Laboratory of Infectious Diseases
LABORATORY OR BRANCH

3. Epidemiology
SECTION UNIT

4. Bethesda
LOCATION

5. NMI -46
SERIAL NO.

6. Epidemiological Studies of Infectious Diseases
PROJECT TITLE

7. Dr. Joseph A. Bell
PRINCIPAL INVESTIGATOR

8. Dr. Robert J. Huebner, Dr. Wallace P. Rowe, Ruth E. Anderson
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

A. Basic epidemiologic studies of undifferentiated respiratory illnesses generally known as the common cold, catarrhal fever, non-streptococcus sore-throat, ARD, etc.

Objectives:

To discover etiologic agents, methods of identification, modes of spread, and methods for prevention and treatment.

Methods Employed:

Person to person spread viral diseases are of special interest because tissue culture techniques now open the door for intensive virus study. Acute febrile diseases are being observed in Military Groups (Great Lakes Naval Training Center); in civilian population groups (20,000 persons of Influenza Study, q.v.); in children 2 months to 12 years of age (District of Columbia orphanage); in hospital admissions (Clinical Center, D. C. General Hospital, and Children's Hospital); and in other groups (schools, camps, et al) which come to light through notification by private physicians and Health Officials. Clinical and epidemiologic data, and many specimens for laboratory study are collected from persons with acute febrile disease and their non-ill associates (controls). These are indexed, coded, and tabulated in a multitude of ways to find groups of cases which epidemiologically appear to be caused by a single infectious agent. Specimens from these cases are then subjected to intensive laboratory study in an effort to find a common agent. When successful, an intensive epidemiologic study is set up to establish etiology and to identify the clinical and epidemiologic characteristics of the disease and to devise methods for control.

Volunteers at the Federal Reformatory in Chillicothe, Ohio, and the State Reformatory at Breathedsville, Maryland, are inoculated with various viruses and other material to better elucidate host parasite relations of human infection, immunity and disease. This limited approach has been particularly profitable in promptly giving answers to important questions which otherwise might take years to obtain.

Major Findings:

The cooperating virus laboratory discovered new viruses of the respiratory tract in 1953 and with the help of the above epidemiological setup identified the group and type characteristics of APC viruses. In 1954, through the hospital setup, Type 3 APC virus was found in six cases of acute respiratory disease, some having conjunctivitis. Immediately, an epidemiologic study was arranged which established that a newly recognized disease entity, Pharyngoconjunctival Fever, was caused by Type 3 virus, (Published 3/55 J.A.M.A.) and abundant clinical, pathological, and epidemiological information was obtained. This led to studies, in volunteers, which developed clinical, epidemiological, and laboratory methods for readily recognizing human susceptibility, immunity, infection and disease. An inactivated virus vaccine was prepared and shown to protect volunteers against infection and disease. (Published November, 1955, J.A.M.A.)

Through studies of cases at the Great Lakes Naval Training Center, APC Types 3, 4, and 7, viruses have been confirmed as a major cause of acute febrile respiratory diseases in military recruits. Thus a polyvalent inactivated APC virus vaccine Types 3, 4, and 7, has been prepared and is being studied to determine its effectiveness in preventing the naturally occurring infections and disease in military recruits.

Proposed Course of Project:

The epidemiologic techniques that have been developed and new laboratory tools available hold promise for further discovery of the cause and clinical epidemiological attributes of other at present undifferentiated respiratory diseases. It is proposed to continue the project for these ends. It is further proposed to develop the present promising hypotheses of vaccine control of some of these diseases. In addition it is proposed to explore other interesting leads as to possible virus causes of other human ailments.

B. Epidemiological study of host parasite relations of poliomyelitis has been discontinued.

Although it was known that prior tonsillectomy and current pregnancy were associated with severe attacks of poliomyelitis, the studies carried out by this section produced data which indicated that tonsillectomy or conditions which prompt this operation and that pregnancy not only increased the severity of attack but also increased susceptibility to attack. (Published September 27, 1955, Annals of the New York Academy of Sciences).

Budget Data Sheet

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BUDGET ACTIVITY:

RESEARCH

13. ______________________

IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATION PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957

None

14. ______________________

IF THIS PROJECT RESEMBLES, COMPLEMENTS OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PHS (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS) IDENTIFY SUCH RESEARCH.

None

Honors, Awards, and Publications Sheet

15.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

Pharyngoconjunctival Fever -- Epidemiological Studies of a Recently Recognized Disease Entity

Joseph A. Bell, M.D., Wallace P. Rowe, M.D., Joseph I. Engler, M.D., Robert H. Parrott, M.D., and Robert J. Huebner, M.D.

Studies of Adenoidal-Pharyngeal-Conjunctival Vaccines in Volunteers

Robert J. Huebner, M.D., Joseph A. Bell, M.D., Wallace P. Rowe, M.D.
Thomas G. Ward, M.D., Baltimore, R. Gerald Suskind, M.D.
Janet W. Hartley, M.S., and Ralph S. Paffenbarger, Jr., M.D.

17. HONORS OR AWARDS FOR THE PROJECT

None

R. P. C. - 1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

1. National Microbiological Institute
INSTITUTE

2. Laboratory of Infectious Diseases
LABORATORY OR BRANCH

3. Experimental Therapeutics Section
SECTION

4. Bethesda
LOCATION

5. NMI- 48
SERIAL NO.

6. Nutrition and Metabolism of Mammalian Cells
PROJECT TITLE

7. Dr. Harry Eagle
PRINCIPAL INVESTIGATOR

8. Dr. Stanley Barban, Dr. Norman P. Salzman, Dr. Karl Habel, Dr. Albert J. Dalton, Jr. (Cancer Institute), Dr. Karl A. Piez (Dental Institute), Dr. Leon Levintow (Cancer Institute), Dr. George Foley (Harvard Medical School)
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

A. The nutrition and metabolism of mammalian cells (Eagle).

Objectives:

To define the nutritional requirements of mammalian cells in tissue culture, with particular reference to the differences, if any, between normal and malignant human cells.

Major Findings:

(1) Continuing the studies initiated last year, 7 vitamins (choline, folic acid, nicotinamide, pantothenate, pyridoxal, riboflavin, and thiamin) have been found to be essential for the survival and growth of the mouse fibroblast and of the HeLa cell. Specific vitamin deficiencies could be produced in either cell by the omission of any one vitamin; and the resulting deficiency state could be cured by the restoration of the missing component to the medium.

(2) In collaboration with Dr. Albert J. Dalton, Jr., of the National Cancer Institute, studies are in progress on the structural changes produced in these cells by various vitamin deficiencies, as visualized by phase and electron microscopy.

(3) The amino acid requirements of two additional human cell lines have been determined: an epidermoid carcinoma of the jaw (strain KB), isolated in this laboratory, and a strain of monocytic leukemia cultured from the circulating blood by Osgood. Qualitatively and quantitatively, the amino acid requirements of these 2 cell lines resemble those previously found for the HeLa and L cells.

(4) The electrolyte requirements of the HeLa cell and of the mouse fibroblast have been defined. These include Na+, K+, Cl^-, HPO_4, Ca++ and Mg++.

Proposed Course of Project:

(1) The determination of the amino acid and vitamin requirements of a number of normal human cell lines; the exploration of possible differences between the growth requirements of normal and malignant cells.

(2) To explore the nutritional function of the serum protein required by all cells so far studied, over and above the 13 essential amino acids and the 7 essential vitamins.

B. Metabolic pathways of cells in tissue culture (Barban).

Objectives:

To study the metabolic patterns of normal and malignant mammalian cells in tissue culture.

Major Findings:

It has been found that a mouse fibroblast cell (strain L) and a human uterine carcinoma cell (strain HeLa) can synthesize most of the non-essential amino acids through transamination reactions. It has also been possible to demonstrate transaminase reactions involving glutamine, an essential metabolite of these cells. While no absolute qualitative differences between the enzyme patterns of the two cell lines have emerged, evidence has been obtained of certain quantitative differences.

The HeLa cell has been found to have a high rate of oxidative metabolism. All the reactions and enzymes of the citric acid cycle have been demonstrated.

Significance to Microbiological Research:

(1) The metabolic patterns of normal and malignant cells provide an approach to the interpretation of nutritional requirements.

(2) Transamination studies are important in an understanding of the mechanisms involved in the biosynthesis and interconversion of amino acids required for the growth of tissue cells.

(3) The finding of all the enzymes of the Krebs citric acid cycle is believed to be the first demonstration of the complete cycle in a single mammalian carcinoma cell.

Proposed Course of Project:

(1) To complete the comparative study on the scope of transamination reactions in the mouse fibroblast and carcinoma cell, and to follow up any notable differences found.

(2) To study enzyme synthesis in normal and malignant cells grown in tissue culture.

C. Nucleic acid synthesis by mammalian cells in tissue culture

(Salzman, Eagle, Levintow)

Objectives:

To elucidate the pathways by which ribonucleic acid (RNA) and desoxynucleic acid (DNA) are synthesized by human cells in tissue culture.

Major Findings:

The incorporation of radioactive precursors (glutamine-2-C^{14}-amide-N^{15}) into nucleic acids has been studied. It has been found that glutamine carbon is utilized as a building block for both the purines and pyrimidines of RNA. The fact that it is utilized four times more effectively as a pyrimidine precursor indicates the reaction sequence oxalacetate aspartate ureido succinic pyrimidines as a possible pathway.

The small degree of dilution of radioactivity in the pyrimidines relative to the glutamine precursor, in spite of the presence of large quantities of glucose in the medium, indicates that relatively little glucose is utilized via the tricarboxylic acid cycle.

Significance to Microbiological Research:

Mammalian tissue in an actively growing state represents a unique system ideal for a study of DNA synthesis. It is hoped that when routes of DNA and RNA synthesis in tissue cultures are understood, studies can be carried out with virus-infected tissue cultures. The biochemical alteration of DNA and RNA synthesis in the host cells by virus is of obvious and central significance in relation to the pathogenesis of viral infection and the mechanism of viral formation.

Proposed Course of Project:

(1) The utilization of C^{14}-labeled aspartic and ureido succinic acid for pyrimidine biosynthesis will be studied in order to elucidate the exact pathways involved.

(2) Precursors for cellular DNA will be studied.

(3) The role of the essential amino acids in purine biosynthesis will be examined.

(4) It is also intended to explore the possibility that RNA may serve as a precursor of DNA. The routes of synthesis of nucleic acids by normal and malignant tumor cells will be compared.

D. The metabolic pathways of viral synthesis (Eagle and Habel).

Objectives:

To determine the components of the medium necessary to viral synthesis, and their metabolic role.

Major Findings:

In a model system, consisting of the HeLa cell infected with poliomyelitis virus, the only components of the medium necessary for the production of optimal amounts of virus were salts, glucose, and glutamine. In the absence of glucose and glutamine, only minimal amounts of virus were produced even when the medium contained the 18 other amino acids, all the essential vitamins, salts, proteins, purines and pyrimidines. Conversely, in a medium consisting only of salt solution, glucose and glutamine, normal amounts of virus were formed.

Proposed Course of Project:

(1) To extend these observations to a number of other cells and virus systems. (Eagle and Habel)

(2) To study the effect of viral infection on the transaminating activity of HeLa cells. (Barban)

(3) To determine the effect of vitamin deficiency on the virus-producing capacity of the cell. (Eagle)

(4) To determine the metabolic function of glucose and glutamine in viral synthesis. (Eagle, Salzman, DeMars, Barban)

E. Protein synthesis, amino acid exchange and protein turnover in tissue culture (Eagle and Piez).

Objectives:

To study the amino acid composition and protein composition of mammalian cells in tissue culture as affected by the environment; and to study the incorporation of isotopically labeled amino acids into protein by non-growing and growing cells, as an approach to the problems of amino acid exchange and protein turnover.

Major Findings:

The HeLa cell has been found to have a large amino acid pool, the magnitude of which is unaffected by prolonged starvation. Labeled amino acids have been found to be actively incorporated into cellular protein. Surprisingly, there was a large degree of incorporation even when the cells were placed in a medium deficient in essential amino acids, and in which there was no net synthesis of protein. The degree of this incorporation has been defined, but its mechanism is not yet clear.

Proposed Course of Project:

Three major possibilities suggest themselves for study.

(1) In bacteria, Gale believes that he has demonstrated that amino acid residues of the amino acid pool exchange with the intact protein molecule, and without having the protein molecule break down completely.

(2) The second possible explanation for the present observations is that there is an actual turnover of protein, with degradation and resynthesis of the protein molecule in the absence of net growth.

(3) In bacteria, Monod has shown that there is no appreciable turnover of protein. The possibility must therefore be considered that there is in fact neither amino acid exchange nor protein turnover, but instead that the incorporation of labeled amino acids in a non-growing medium reflects the death and dissolution of some of the cells, the components of which enter into the protein precursor pool and are used for the synthesis of new cells.

F. The cytotoxic action of carcinolytic agents in tissue culture (Eagle and Foley).

Objectives:

To study the cytotoxic action in tissue culture of carcinolytic agents, with the dual purpose of (a) studying their modes of action, and (b) exploring the possibility of using tissue culture as a primary screen for the discovery of carcinolytic agents.

Major Findings:

A simple chemical procedure has been developed for the determination of cell growth. This greatly facilitates and simplifies the measurement of the cytotoxic activity of various compounds. In collaboration with Dr. George Foley of the Children's Cancer Research Foundation (Harvard Medical School), it has been shown that every one of the carcinolytic agents so far studied is highly cytotoxic in vitro, the effective concentrations ranging from 10^{-2}M to 10^{-9}M. If this can be confirmed in a larger series, there is in hand a simple and quantitatively reproducible method of testing large numbers of compounds for cytotoxic action, reserving the laborious and costly animal screening for those compounds found to be cytotoxic in vitro.

Budget Data Sheet

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12. BUDGET ACTIVITY:

RESEARCH

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATION PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 OR 1957

A. National Cancer Institute

C. National Cancer Institute

E. National Institute for Dental Research

F. Children's Cancer Research Foundation, Harvard Medical School, Boston, Massachusetts

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PHS (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS) IDENTIFY SUCH RESEARCH.

No.

R.P.C. - 3 Analysis of NIH Program Activities SERIAL NO. NMI-48
December 1955

Honors, Awards, and Publications Sheet

15.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

A. Utilization of dipeptides by mammalian cells in tissue culture. Harry Eagle. Proc. Soc. Exp. Biol. Med., 89: 96-99, 1955.

The specific amino acid requirements of a mammalian cell (strain L) in tissue culture. Harry Eagle. J. Biol. Chem., 214: 839-852, 1955.

The specific amino acid requirements of a human carcinoma cell (strain HeLa) in tissue culture. Harry Eagle, J. Exp. Med., 102: 37-48, 1955.

Propagation in a fluid medium of a human epidermoid carcinoma, strain KB. Harry Eagle. Proc. Soc. Exp. Biol. Med., 89: 362-364, 1955.

Nutrition needs of mammalian cells in tissue culture. Harry Eagle. Science, 122: 501-504, 1955.

The growth requirements of two mammalian cell lines in tissue culture. Tr. Assn. Am. Phys., 68: 78-81, 1955. Harry Eagle.

The minimum vitamin requirements of the L and HeLa cells in tissue culture, the production of specific vitamin deficiencies, and their cure. Harry Eagle. J. Exp. Med., 102: 595-600, 1955.

In press:

The growth response of mammalian cells in tissue culture to L-glutamine and L-glutamic acid. Harry Eagle, Vance I. Oyama, Mina Levy, Clara L. Horton, and Ralph Fleischman. J. Biol. Chem.

The salt requirements of mammalian cells in tissue culture Harry Eagle. Arch. Biochem. & Biophys.

B. None

C. None

D. None

E. Systematic effect of C^{14}-labeling on ion-exchange chromatography of amino acids. K. A. Piez and Harry Eagle. Science, 122: 968-969, 1955.

F. None

17. HONORS OR AWARDS FOR THE PROJECT:

None

R. P. C. - 1 Analysis of NIH Program Activities
December 1955

Project Description Sheet

1. National Microbiological Institute
INSTITUTE

2. Laboratory of Infectious Diseases
LABORATORY OR BRANCH

3. Experimental Therapeutics Section

4. Bethesda
LOCATION

5. NMI- 49
SERIAL NO.

6. Mode of Action of Aureomycin
PROJECT TITLE

7. Dr. Arthur K. Saz
PRINCIPAL INVESTIGATOR

8. None
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives:

To determine the biochemical basis for the inhibition of sensitive bacteria by aureomycin and to determine the biochemical mechanism of resistance of bacteria to the antibiotic.

Methods:

Attempts are continuing to isolate, resolve and purify the organic nitro reductases isolated from both sensitive and resistant *E. coli*.

Major Findings:

It had previously been found that the cell-free nitro reductase isolated from aureomycin-resistant *E. coli* is resistant to aureomycin. Comparison of this highly purified enzyme with that isolated from the sensitive strain indicates that both enzymes are flavoproteins. The resistant enzyme tenaciously binds its conjugated flavin, while the sensitive enzyme is easily dissociated into its flavin component and its apoenzyme. By fluorescence determinations, it has been found that the resistant enzyme contains 15 to 20 times more flavin mononucleotide per mg. protein than the sensitive extract. In contrast to the sensitive reductase, which requires Mn^{++} or Mo^{+6}, it has proved extremely difficult to show a cation requirement for the resistant enzyme. However, Mo^{+6} stimulates the resistant nitro reductase 15 to 20 percent. It is therefore postulated that the antibiotic inhibits the sensitive enzyme by virtue of its ability to compete successfully with the enzyme for the easily dissociated essential cation, but is unable similarly to remove the cation from the resistant enzyme.

A number of other electron transport enzymatic systems (including ferricyanide and phenol-indophenol reductions) are inhibited by the antibiotic. The reduction of the yellow, highly purified resistant enzyme by DPNH is inhibited by high concentrations of aureomycin to the same extent as is the nitro reductase. The sensitive enzyme does not contain enough flavin to permit the ready following of the electron flow through it.

From the above considerations it is postulated that aureomycin is active by virtue of its ability to inhibit the electron transport system and consequently the energy requirements of sensitive cells, and that the development of resistance reflects the presence of an altered enzyme.

Significance to Microbiological Research:

Studies on the mode of action of antibiotics and on mechanisms of development of resistance to these drugs provide a rational approach to the chemotherapy of infectious diseases.

Proposed Course of Project:

It is proposed to study more definitively the electron transport systems of sensitive and resistant cells in order to determine precise differences between these two classes of organisms.

Budget Data Sheet

10.

11.

12. BUDGET ACTIVITY:

RESEARCH

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATION PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 OR 1957

None

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PHS (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS) IDENTIFY SUCH RESEARCH.

None

Analysis of NIH Program Activities

SERIAL NO. NMI-49

Honors, Awards, and Publications Sheet

15.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

None published

In press:

Aureomycin-resistant cell-free nitro reductase from aureomycin-resistant *Escherichia coli*. Arthur K. Saz, Lee W. Brownell, and Rita B. Slie, J. Bact.

17. HONORS OR AWARDS FOR THE PROJECT:

None

R.P.C. 1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

1. National Microbiological Institute
INSTITUTE

2. Laboratory of Infectious Diseases
LABORATORY OR BRANCH

3. Virus and Rickettsial Diseases
SECTION

6. Virus therapy of cancer
PROJECT TITLE

4. Bethesda
LOCATION

5. NMI - 50
SERIAL No.

7. Dr. R. J. Huebner
PRINCIPAL INVESTIGATOR

8. Dr. W. P. Rowe, Dr. R. Gerald Suskind, and Miss Janet Hartley
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

A. Studies in Humans

Objectives:

To test in cancer patients the anti-neoplastic effect of mildly pathogenic viruses which are oncolytic against cancer cells grown in tissue culture (in vitro).

Methods Employed:

There are some 13 APC (adenoidal-pharyngeal-conjunctival) viruses, each immunologically distinct from the other, which propagate and readily destroy human carcinoma cells grown in vitro. These viruses, when encountered in naturally occurring disease, or when administered to human volunteers, produce minor respiratory and ocular diseases. These illnesses do not occur unless the viruses gain entrance initially into the mucous membranes of the conjunctiva or the upper respiratory tract. When large amounts are introduced by other routes, virtually no disease results.

Virus preparations of various types, showing high titers and filtered to remove live HeLa cells, and tested for human pathogens other than the APC's,are made available for inoculation into cervical carcinoma cases, which is done in collaboration with the National Cancer Institute (Dr. Robert Smith). Thus far, cancer patients having late stage (stage 3 or 4) carcinoma of the cervix, have been selected for inoculation with these oncolytic viruses. Their serum is pretested for specific neutralizing antibodies against the various immunological types. Those types of virus for which the patient does not have neutralizing antibody, are then considered for administration to the patient, either locally and directly into the tumor or by means of inoculation into the arterial supply of the pelvic area. The patient is followed intensively for any clinical and laboratory evidence of virus infection or other generally deleterious effects. Blood specimens and specimens from the throat, rectum, urine, and particularly from the cervical tumor area are taken at frequent and regular intervals

Methods Employed (continued):

to be tested for the inoculated virus. Antibody studies are done as well, to determine the immunological response of the patient to the virus, particularly to determine the rapidity with which neutralizing antibody develops. APC viruses, isolated from tumor tissue showing oncolysis, are selected for further propagation and for consideration as inocula for additional cancer patients.

Since the rapid and very effective antibody response of the patient to the inoculated virus quickly terminates viral activity in the area inoculated, several methods have been employed for suppressing antibody responses. Large doses of cortisone given before and during the period of virus infection, have been used in an effort to accomplish this.

Major Findings:

To date a total of 34 patients, most of them with far advanced cancer, and some in terminal stages, have been studied. Twenty-three cases have received local injections with either active virus or control material, and 11 have received the active virus injected intra-arterially. Of the 12 receiving local injections of 1 to 50 ml. of live virus, 10 showed definite necrosis of the lesion following injection. None of the 11 receiving control material, which consisted of either normal HeLa culture fluid, heat inactivated virus, or active virus plus antibody, showed a similar clinical result. Seven patients, six of whom had received large doses of cortisone, were injected intra-arterially with suspensions of the virus. Three of the seven showed definite necrosis of the lesion. A small number (4) were given both intra-arterial and local virus injections on the operating table. All four showed definite necrosis.

Laboratory studies have shown that virus can be recovered from the carcinomatous lesion for varying periods of time following injection. Cortisone appeared to increase the number of isolations beyond 10 days or more after inoculation, and in one instance, considerable amounts of virus were shown to be present in the secretions from a necrosing tumor 17 days after primary inoculation. A number of patients having received one fairly large inoculation of virus (several hundred ml.), were inoculated with an equivalent amount, several weeks later, of another immunological type. In several cases, extensive necrosis followed each inoculation.

Despite the definite necrosis produced in a rather large percentage of patients receiving virus, in no instance did this necrosis proceed to elimination of the tumor, and in virtually all cases, regrowth of the tumor at the site of oncolysis was observed. Although several cases might possibly be regarded as experiencing a modification of the disease, in most instances the course of the illness did not appear to be appreciably altered. Since many of the cases were in a terminal stage of illness at the time of inoculation, of course, they have ended as expected, with fatal termination. Pathological studies of the biopsies taken during the periods before, during and after the gross necrosis was observed, have not been particularly helpful in defining

Major Findings (continued):

the site of virus activity. Thus, it is not known at the present time whether or not the necrosis which occurs in the cervical carcinomas is due to a specific viral oncolytic activity upon the cancer cell, or whether the viral activity is occurring in interstitial tissues, producing thrombosis of capillaries serving cancer cells resulting, secondarily then, in destruction of tumors through lack of blood supply.

A few cases, chiefly those inoculated with virus, and also those on cortisone therapy, responded with self-limited febrile episodes, some of which could be attributable to systemic virus infection. The majority of patients showed little or no evidence of infection attributable to the inoculation, and indeed, the virus inoculation did not seem to alter, in any perceptible way, the general state of health.

Significance to Microbiologic and Cancer Research:

The demonstration that prevalent, human viruses, responsible for minor disease in nature, when inoculated into cancer tissues, seldom or never produce evidence of viral disease, and are capable of producing (either directly or indirectly) extensive oncolysis in vivo of carcinoma cells, is, in itself, a notable observation. Although only one group off such viruses has been used thus far, it supports the thesis that viral agents, not particularly pathogenic for normal tissue cells, may be selected or trained to cause a characteristic and specific destruction of carcinomatous tissue. These findings lend encouragement to the notion that the oncolytic effects may be increased by various methods such as preventing antibody responses to inoculated agents, by giving in rapid succession immunologically different agents that may be found or may be traindd to be capable of reaching and destroying all cancer cells in the body.

Proposed Course of Project:

Several lines of investigation are planned.

1. Utilization of larger amounts of cortisone or perhaps other methods for suppressing antibody response to the oncolytic viruses will be tried in an effort to extend virus activity beyond present limits.

2. Laboratory studies of APC viruses show that increased concentrations of virus, even when not greater than 1 to 5 and 1 to 10, may produce profound effects upon the rapidity with which cancer cells are destroyed in vitro. Thus, increasing the viral content 2 to 4 times may result in reducing an incubation of oncolysis in the test tube from five to six days to as little as one or two days. Consequently, it is believed that large amounts of APC viruses, which are concentrated and purified from the original state, should be tried in order to determine whether or not such procedures will result in more rapid and more extensive necrosis of carcinoma. Thus, as much as five to ten liters of virus conceivably could be produced and concentrated into less than 100 ml. of fluid before inoculation. This increased mass of virus may be expected to have two effects -- a) greater dissemination of virus particles through the body when

Proposed Course of Project (continued):

given intra-arterially or perhaps intravenously, and b) overwhelming antigenic stimulus, which if given in repeated doses over a number of days, may have a suppressing effect on antibody production.

3. Improved methods for inoculation and for dissemination of the virus to be injected, such as the addition of spreading factors (hyaluronidase) to the virus inoculum.

4. There are numerous other prevalent viruses of man which are constantly becoming available through the use of new techniques for demonstrating viruses. Many of these appear to be almost non-pathogenic, as judged by the circumstances in which they are encountered, and yet have profound oncolytic effects on carcinoma cells in vitro. It is proposed that these agents also be tested for oncolysis on carcinoma in vivo. The utilization of such agents, of course, will be given following proper examination by the medical board, and initial studies will be carried out by use of very small amounts given locally, directly to the tumor, in the same way that the initial tests of APC viruses were carried out.

B. Studies in human cancer cell grown in animals

Objectives:

To screen the oncolytic effects of numerous viruses, both of human and animal origin on human carcinoma grown in animals (Toolan technique).

Methods Employed:

Weanling rats are pre-treated with whole body irradiation and with cortisone in such a manner as to provide maximum growth of transplanted human tissue. Regular and reasonably uniform tumors are produced via the intraperitoneal inoculation of tissue culture from carcinomatous cells (HeLa and KB tissue culture cells). The size and the progression of the tumor is determined by the number of viable cells (trypsinized) inoculated. Thus, large doses of tissue culture grown cells produce, within seven to ten days, a comparatively large and easily recognizable tumor mass attached, generally, to the omentum. This tumor continues to grow rapidly, and by invading the diaphragm and various other tissues, frequently results in the death of the rat. Since the tumors can be produced regularly, well controlled screening tests can be carried out to detect the oncolytic effects of many viruses upon such tumors given at various stages during the growth of the tumor. Most such viruses are grown first in tissue culture and passaged there, to determine oncolytic activity in vitro and in most instances, also, to increase the adaptation of the virus to the cell type and to shorten the incubation period required for oncolysis. Similarly, the viruses showing some activity in vivo on the human tumor grown in the rat, will be selectively passages in this system in efforts to increase the oncolytic effects. Those agents showing greatest promise, in other words,

Methods Employed (continued):

producing the greatest amounts of oncolytic effects, will then be tested for pathogenic activity in the rat as host and in other laboratory animals before its use in humans will be contemplated.

MAJOR FINDINGS:

Methods as above have been developed. A new project.

Significance to Microbiologic and Cancer Research:

Although the growth of human tumor tissue in laboratory animals such as the rat has been reported and is in widespread use, the regular production in nearly all inoculated animals of large and easily evaluated tumors, represents a laboratory tool and an opportunity for screening anti-neoplastic agents not previously available. Thus, large-scale experiments can be designed to test the effects of anti-cancer chemotherapeutic agents as well as numerous human and animal viruses. In the search for anti-cancer agents, the ideal material would represent an agent capable of destroying cancer cells, with little or no toxic effects upon normal tissue. Most viruses have developed through long, evolutionary experiences and show a high degree of specificity for cell types, which is seldom encountered in non-living chemical substances. Because of the extremely high reproductive rate of the viruses, this evolutionary process can be, to a certain extent, directed in such a way that lytic effects can be increased for certain cells, while at the same time, they are decreasing for others. This is a commonly used biological procedure which has met with considerable success in modifying viral agents which are contemplated for use in viral vaccines.

Proposed Course of Project:

This is a new project and the direction it will take is described under methods.

Budget Data Sheet

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12. BUDGET ACTIVITY:

Research

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATION PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957.

Dr. Robert R. Smith, and associates - Surgery Branch, NCI

Dr. Harold Stewart, Dr. Louis B. Thomas, and associates - Surgical Pathology Service, NCI

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PHS (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS) IDENTIFY SUCH RESEARCH.

None

Honors, Awards, and Publications Sheet

15.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955.

NONE

17. HONORS OR AWARDS FOR THE PROJECT

None

R. P. C. - 1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

1. National Microbiological Institute
INSTITUTE

2. Laboratory of Infectious Diseases
LABORATORY OR BRANCH

3. Virus and Rickettsial Diseases
SECTION

4. Bethesda
LOCATION

5. NMI - 51
SERIAL NO.

6. Study of a Virus Possibly Representing the Salivary Gland Virus of Humans.
PROJECT TITLE

7. Dr. Wallace P. Rowe
PRINCIPAL INVESTIGATOR

8. Miss Janet W. Hartley, Dr. Robert J. Huebner
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives:

To determine the relation of a virus recently isolated in this laboratory, thought to represent the human salivary gland virus, to human disease and to the salivary gland viruses of animals.

Methods Employed:

Tissue culture procedures have made possible, for the first time, the isolation of viruses thought to represent the human salivary gland virus. Tissue culture and serological procedures will be used chiefly for the studies of the human strains. In parallel studies of animal salivary gland viruses, a limited number of small laboratory animals will be used, although it is anticipated that tissue culture techniques can be developed for these strains as well.

Major Findings:

The presence of viral inclusion bodies in the salivary glands of many mammals, including humans, has been recognized for many years but until recently, little progress has been made in their study. Recently, viruses thought to represent the human salivary gland virus were isolated independently in St. Louis, Boston, and this laboratory. A strain recovered here has been adapted to rapid growth in tissue culture of human fibroblasts and is the only strain on which adequate virological studies can be performed. With this strain, a complement fixation test has been developed for the first time, and antibodies found to be almost universally present in adult humans.

Significance to Microbiologic Research:

Pathological evidence suggests that the human salivary gland virus, in addition to causing the rare but highly fatal illness, cytomegalic inclusion body disease of infants, may represent an important cause of abortion, stillbirth, and infant pneumonitis, as well as producing secondary infections complicating other diseases. In view of the generalized visceral involvement in cytomegalic disease, it appears possible that lesser degrees of generalization could result in infection of a wide variety of individual organs, and consequently a number of disease pictures. The high prevalence of antibodies to this virus, as determined by the complement fixation developed in this laboratory, indicates that it may represent a prolific source of human disease.

The development of serological tests also makes it possible for the first time to determine the immunological relation of the various viruses producing salivary gland infection in different species, and should facilitate study of the pathogenesis and epidemiology of these infections.

Proposed Course of Project:

Laboratory techniques for detecting neutralizing antibodies and for isolating the virus must be perfected. Serological surveys of acute and convalescent serums of patients with a variety of infectious diseases of undetermined etiology will be carried out by means of the complement fixation test. At present, this laboratory is the only one in a position to perform this test. This test will also be incorporated into the longitudinal studies of various populations carried out in connection with respiratory disease studies. If a correlation is found with any disease, suitable epidemiological studies will be instituted to determine its role in the condition. The mouse salivary gland virus will be studied in tissue culture, and attempts will be made to perform serological tests to determine its relation to the human virus. In addition, attempts will be made to establish the guinea pig and rat salivary gland viruses in tissue culture for the first time, and similarly determine their interrelationships.

Budget Data Sheet

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12. BUDGET ACTIVITY:

RESEARCH

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATION PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957.

None

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PHS (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS) IDENTIFY SUCH RESEARCH.

None

R. P. C. - 3 Analysis of NIH Program Activities SERIAL NO. NMI- 51
December 1955

Honors, Awards, and Publications Sheet

15.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

None

17. HONORS OR AWARDS FOR THE PROJECT

None

R. P. C. - 1 Analysis of NIH Program Activities
December 1955

Project Description Sheet

1. National Microbiological Institute
INSTITUTE

2. Laboratory of Infectious Diseases
LABORATORY OR BRANCH

3. Virus and Rickettsial Diseases
SECTION

4. Bethesda
LOCATION

5. NMI- 52
SERIAL NO.

6. Development and Application of Techniques
PROJECT TITLE

7. Dr. Leon Rosen
PRINCIPAL INVESTIGATOR

8. Dr. R. J. Huebner, Dr. W. P. Rowe, Miss Janet W. Hartley, Richard Lynt.
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION:

A. Development and application of techniques for rapid identification and characterization of numerous, newly recognized viruses.

Objectives:

To devise simplified, rapid, and generally applicable techniques for identifying newly isolated virus agents and classifying them within proper generic categories.

Methods Employed:

Tissue Culture - To the extent that it proves applicable, attempts to characterize and immunologically identify viral agents will be made in tissue culture. Tissue culture neutralization tests are presently used for identification of some 25 separate viruses falling within polio, APC, Echo and Coxsackie families. Since most of the Coxsackie viruses however, cannot be propagated in tissue culture, methods for working in suckling mice will also be necessary. Similarly, certain other viruses that grow best in chick embryos will undoubtedly have to be dealt with in this medium. Prototype antisera prepared in rabbits, possibly also in monkeys and mice, against the many representatives of these viruses, will be prepared. Research efforts will be directed towards simplifying and telescoping the long-term, almost impossible procedures now necessarily used for identification purposes. Thus, multiple sera prepared from agents falling in the same family groups, will be pooled and their efficiency in detecting representatives of that family determined. Other possible differentiating characteristics such as complement fixing antigens, hemagglutinins, growth characteristics, and tropisms for certain tissues will also be examined with the purpose of devising short cuts to a most necessary and namely, quick identification of viruses.

Major Findings:

During the last five years, this Unit has developed a number of techniques for identifying and classifying many Coxsackie and all of the 12 APC viruses. We have devised methods for distinguishing in tissue culture, other agents such as polio, herpes and vaccinia virus. These methods are efficient and accurate; however, they are time consuming and extremely expensive. Certain short-cuts, such as the use of the complement fixation test in identifying the specific CF antigens of various Group A Coxsackie viruses, have already been tested and shown to be valuable. By utilizing pooled hyper-immune serum paired against as many as five or six APC viruses, it is now possible to obtain quick and rough classifications of large numbers of unidentified agents falling into this group.

Significance to Microbiologic Research:

Virus laboratories in all parts of the world have acquired and are continuing to acquire large numbers of viral isolates which remain unidentified. Even the larger and more experienced of the virus laboratories find it is virtually impossible to keep up with identification of viral agents encountered solely in their own surveys. The very considerable time spent in attempting such identification is, of course, unavailable for more productive studies. However, if these identification procedures are not carried out and if viruses are not correctly classified and identified in early stages of study, much fruitless work follows on known agents, and frequently results in reports which are either redundant or inadequate in the sense that many assumptions and conclusions are drawn from inadequate data; assumptions which may have already been shown to be invalid by previously reported large-scale studies on the same agents. Perhaps of most importance is the fact that many promising viral agents of man are now being studied only indifferently because the laboratories having found them are, at present, unable to determine whether or not they represent new or previously recognized agents about which much is already known. Development of streamlined and yet highly accurate techniques for identification, is, perhaps, one of the most indispensible pre-requisites for maximum progress towards prevention of newly recognized and important viruses of man.

Proposed Course of Project:

Since this is essentially a new undertaking, the course of the project is best indicated above under "Methods".

Budget Data Sheet

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12. BUDGET ACTIVITY:

RESEARCH

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATION PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957.

None

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PHS (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS) IDENTIFY SUCH RESEARCH.

None

R. P. C. - 3 Analysis of NIH Program Activities SERIAL NO. NMI- 52
December 1955

Honors, Awards, and Publications Sheet

15.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

None

17. HONORS OR AWARDS FOR THE PROJECT

None

R. P. C. - 1 Analysis of NIH Program Activities
December 1955

Project Description Sheet

1. National Microbiological Institute
INSTITUTE

2. Laboratory of Infectious Diseases
LABORATORY OR BRANCH

3. Virus & Rickettsial Diseases
SECTION

4. Bethesda
LOCATION

5. NMI- 53
SERIAL NO.

6. Study of Viruses as Causes of the Common Cold and Other Common Respiratory Diseases,
PROJECT TITLE

7. Dr. Wallace P. Rowe
PRINCIPAL INVESTIGATOR

8. Dr. Robert J. Huebner, Dr. Leon Rosen, Miss Janet Hartley, Miss Christine Cummings.
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

A. Continuation of studies of the clinical importance of 12 immunologically distinct APC viruses.

Objectives:

Although 3 of the 12 or more newly recognized APC virus agents are now well established as the cause of specific and very prevalent respiratory illnesses, a proportion of primary atypical pneumonias, and possibly, also, epidemic keratoconjunctivitis, the other 9 viruses remain to be studied with respect to their importance in the production of human respiratory and ocular illnesses.

Methods:

Laboratory techniques for identification, classification and characterization of the APC viruses are now well develcped. These laboratory methods will be applied to epidemiologic, clinical studies and surveys of certain human populations in which the APC viruses are known to be prevalent. Both longitudinal and cross-section studies of sample populations, selected on household, school, or institutional basis are planned. Volunteer studies, as indicated, will also be carried out in collaboration with the U.S. Bureau of Prisons. The requirements of studies of this sort are considerable. Literally thousands of specimens will be collected and tested in various types of tissue culture systems for the presence of APC viruses. The results of findings on these specimens then must be related to their distribution amongst the ill and well segments of the populations under study.

Major Findings:

The original group of six agents in the APC family was increased during 1955, to a total of 12 human types plus several animal representatives. The clinical significance of these agents in respiratory and ocular disease was greatly extended. APC Types 1, 2, and 5 were found to be almost universally prevalent in young children, Type 2 occurring most frequently before age 6. These 3 representatives have been associated with prevalent febrile illnesses, characterized by pharyngitis, frequently by otitis media, bronchitis, etc., but their etiologic importance is not yet fully evaluated.

APC 3 was demonstrated to be the specific cause of extensive outbreaks of pharyngoconjunctival fever, occurring in summer school and summer camp groups in which swimming in a lake or a pool was incriminated as a possible factor in the spread of disease. Frequent sporadic cases also occur throughout the year, without such exposure. Type 4 and Type 7 have been confirmed by our group as well as others, as a major cause of "ARD", the extremely prevalent acute respiratory disease of military recruits.

Type 8 has been associated by Jawetz and Thygeson in California, with epidemic keratoconjunctivitis, a severe and serious disease of the cornea which has been epidemic in many parts of the world, causing high morbidity among shipyard workers in the United States during World War II. This observation must be confirmed.

The other serotypes have been encountered only occasionally, generally in relation to respiratory or conjunctival illness. Studies in volunteers, in collaboration with the Virus Department of the Johns Hopkins School of Hygiene and Public Health, resulted in the development of a method for regularly reproducing illnesses similar to pharyngoconjunctival fever in volunteers now having pre-existing antibodies to the viral types inoculated. This provided an opportunity for testing a monovalent Type 3 APC vaccine which successfully prevented experimental illness.

Significance to Microbiologic Research:

The APC group of viruses, reported initially from this laboratory in 1953, represents a large group of immunologically related but clinically heterogeneous human viruses. At least 3 clinically distinct respiratory diseases* have been well associated with some of the types and preliminary information indicates other types may be responsible for equally prevalent illnesses. This group of agents appears to account for a significant proportion, but not the majority, of the so-called undifferentiated respiratory diseases. Furthermore, a number of external diseases of the eye, such as epidemic keratoconjunctivitis, catarrhal and follicular conjunctivitis, appear to be caused by certain APC viruses.

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* ARD (acute respiratory disease), pharyngoconjunctival fever, and febrile pharyngitis.

Proposed Course of Project:

This Section will continue to collaborate with colleagues in other laboratories of NMI in determining as precisely and definitely as possible, the clinical importance of the 12 known APC viruses. Since many other virus laboratories are working on this group of agents, numerous immunological representatives of APC viruses must be typed. At the present time, this laboratory represents the only one in which newly isolated strains of the APC group can be identified and characterized.

B. Studies of Respiratory Diseases of Unknown Etiology.

Objectives:

To continue to search for additional, as yet unrecognized respiratory viruses responsible for common respiratory illnesses other than APC; especially the common cold.

Methods Employed:

1. Clinical material will be collected from naturally occurring illnesses, and volunteers with induced common colds.
2. These materials will be passed through volunteers since experimental animals other than the chimpanzee are known to be insusceptible to agents of minor respiratory illness. For this purpose, larger scale studies amongst prison volunteers will be required; studies designed to include adequate numbers of test and control groups to provide evidence of the highest order concerning the presence or absence of a transmissable agent in the test materials.
3. The clinical material will also be inoculated into tissue cultures of a variety of cell types. It is well known that many viruses are highly specific in their cellular affinities, some capable of infecting only one cell type, and perhaps, accomplishing this only after considerable numbers of blind passages. Thus, it is planned that numerous specimens from suitable cases will be inoculated into a variety of tissue culture cells, including monkey kidney

cells, HeLa cells, normal human and animal epithelial cells, and various fibroblastic cells.

Major Findings:

In addition to demonstration of the various APC viruses, a number of other viruses have been recovered from the respiratory tracts of children and adults undergoing respiratory diseases. Although it is not known that any one of these agents represents the cause of respiratory diseases, one or more of them may indeed have such pathogenic capacity. Thus far, in this as in other laboratories, an agent specifically producing severe coryzal symptoms without fever, an entity usually called the common cold, has not been demonstrated.

Significance to Microbiologic Research:

Although the known agents which cause respiratory disease, such as streptococci, influenza, and more recently the APC viruses, can be shown to cause a large proportion of respiratory illnesses in study populations, it is quite obvious that a very large proportion of these illnesses remain to be identified from an etiological standpoint. The great importance of these infections in causing high morbidity and economic loss has been emphasized so well that it need not be enlarged upon here.

Proposed Course of Project:

The increased proficiency in working with the new virological techniques that have been developed in the last few years, will allow for an intensification and an extension of the search for viral agents which are potential causes of respiratory disease. As new agents are encountered, it is necessary that they be compared with numerous other new agents already described in the literature during the last few years. It is necessary, therefore, to rule out the relationship of newly isolated agents to perhaps 50 other viruses which are known not to cause common colds, before specific studies to determine their relationship to the common cold are justified. However, when an agent which cannot be classified in these known categories is encountered in relation to respiratory disease, laboratory methods for easily recognizing its presence in humans will then be developed and applied to suitable epidemiological and clinical studies in volunteers and in the general population.

Budget Data Sheet

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BUDGET ACTIVITY:

RESEARCH

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATION PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957.

1. Dr. Thomas G. Ward, Lobund Institute, University of Notre Dame
2. Children's Hospital Research Foundation, Washington, D.C.
3. U.S. Bureau of Prisons, U.S. Reformatory for Men, Hagerstown, Md.
4. NAMRU #4

14.

IF THIS PROJECT RESEMBLES, COMPLEMENTS OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PHS (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS) IDENTIFY SUCH RESEARCH.

Honors, Awards, and Publications Sheet

15.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

1. Pharyngoconjunctival fever: epidemiological studies of a recently recognized disease entity. Joseph A. Bell, Wallace P. Rowe, Joseph I. Engler, Robert H. Parrott, and Robert J. Huebner. J.A.M.A., Vol. 157, pp. 1083-1092, March 26, 1955.

2. Studies of the adenoidal-pharyngeal-conjunctival (APC) group of viruses. Wallace P. Rowe, Robert J. Huebner, Janet W. Hartley, Thomas G. Ward, and Robert H. Parrott. Am. J. Hyg., Vol. 61, pp. 197-218, March, 1955.

3. Production of pharyngoconjunctival fever in human volunteers inoculated with APC viruses. Thomas G. Ward, Robert J. Huebner, Wallace P. Rowe, Ralph W. Ryan, and Joseph A. Bell. SCIENCE, Vol. 122, No. 3179, pp. 1086-1087, December 2, 1955.

4. Studies of adenoidal-pharyngeal-conjunctival vaccines in volunteers. Robert J. Huebner, Joseph A. Bell, Wallace P. Rowe, Thomas G. Ward, R. Gerald Suskind, Janet W. Hartley, and Ralph S. Paffenbarger, Jr. J.A.M.A., Vol. 159, pp. 986-989, November 5, 1955.

17. HONORS OR AWARDS FOR THE PROJECT

None

R. P. C. - 1 Analysis of NIH Program Activities
December 1955

Project Description Sheet

1. National Microbiological Institute
INSTITUTE

2. Laboratory of Infectious Diseases
LABORATORY OR BRANCH

3. Virus and Rickettsial Diseases
SECTION

4. Bethesda
LOCATION

5. NMI - 54
SERIAL NO.

6. APC Vaccine Studies
PROJECT TITLE

7. Dr. Robert J. Huebner
PRINCIPAL INVESTIGATOR

8. Dr. W. P. Rowe, Miss Janet Hartley
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

A. Field trials of polyvalent Adenoidal-Pharyngeal-Conjunctival (APC) vaccines designed to prevent prevalent respiratory diseases.

Objectives:

To prepare effective prophylactic vaccines against the large and undetermined proportion of ARD (acute respiratory disease) and other common respiratory and eye diseases which are caused by APC viruses.

Methods Employed:

Several polyvalent vaccines, inactivated with formalin, will be prepared in collaboration with commercial drug houses. The virus for vaccines will be grown in monkey kidney tissue, various immunologic types being combined according to their clinical importance in the population to be protected. Thus, Types 3, 4, and 7 are included in a vaccine for military groups; a vaccine which will be given a field trial in several thousands of military recruits. Another vaccine containing Types 1, 2, 3, and 5 will be studied for effectiveness in pediatric and family study populations, where those types are highly prevalent. Vaccines containing at least 7 separate immunologic types will also be prepared and their effectiveness in producing neutralizing serum antibodies will be measured.

Major Findings:

A monovalent APC 3 vaccine was prepared in monkey kidney tissue cultures and found capable of preventing pharyngoconjunctival fever experimentally induced in volunteers. This successful test of the first respiratory viral vaccine since influenza, was made possible by the adaptation of various APC viruses to monkey kidney tissues directly from the human host materials and the demonstration that formalin in adequate amounts readily inactivated the live viruses without affecting the immunizing antigen deleteriously. The opportunity for quickly and effectively testing the vaccine was presented by the development of methods for reproducing objective illness in susceptible human volunteers.

Significance:

APC viruses Type 3, 4 and 7, have been shown to cause a large proportion, perhaps a majority of acute respiratory disease occurring in military populations - particularly in military recruits. Type 3 is the cause of outbreaks and sporadic cases of pharyngoconjunctival fever, currently very common in the general population. Types 1, 2, and 5 each occur as extremely common infections associated with similar illnesses in infants and young children. The exact clinical importance of these types has not been studied as extensively as the others and remains to be evaluated, but human volunteer studies show them to be pathogenic, and serologic surveys suggest that they cause a large amount of febrile sore throat in children. It can be expected, now that a protective vaccine against these agents has been produced and found feasible, that a general large-scale demand may develop soon.

Proposed Course of Project:

Studies of effectiveness of vaccines vs. APC Types 3, 4 and 7, are planned in collaboration with the Epidemiologic Section of LID, and military research groups (NAMRU #4), and the Virus and Rickettsial Laboratory of the California State Department of Public Health.

Additional vaccines vs. APC Types 1, 2, 3 and 5 will be prepared, and tested for potency and effectiveness in field studies designed along the lines of present influenza vaccine studies now being carried out by the Epidemiologic Section. Control procedures for maximum safety and potency, applicable to large scale production and use in general populations, will be developed in collaboration with the Division of Biologics Standards.

Budget Data Sheet

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12. BUDGET ACTIVITY:

RESEARCH

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATION PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 OR 1957.

a. NAMRU #4, Great Lakes Naval Training Station, Great Lakes, Ill.

b. Virus & Rickettsial Laboratory, California State Department of Public Health.

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PHS (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS) IDENTIFY SUCH RESEARCH.

Honors, Awards, and Publication Sheet

15.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

1. Studies of adenoidal-pharyngeal-conjunctival vaccines in volunteers. R. J. Huebner, Joseph A. Bell, Wallace P. Rowe, Thomas G. Ward, R. Gerald Suskind, Janet W. Hartley, and Ralph S. Paffenbarger, Jr. J.A.M.A., Vol. 159, pp. 986-989, November 5, 1955.

17. HONORS OR AWARDS FOR THE PROJECT

None

Project Description Sheet

1. National Microbiological Institute
INSTITUTE

2. Laboratory of Tropical Diseases
LABORATORY OR BRANCH

3. Office of the Chief
SECTION

4. Bethesda
LOCATION

5. NMI - 55
SERIAL NO.

6. Administration and Research Planning and Coordination
PROJECT TITLE

7. Dr. Willard H. Wright
PRINCIPAL INVESTIGATOR

8. Dr. G. Robert Coatney
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

This project furnishes certain technical, supervisory, and administrative services to all research projects in the Laboratory, as follows

(a) Over-all planning of research, the coordination of research activities in the various Sections of the Laboratory, and the direction of Foreign Field Stations.

(b) Integration of Laboratory research activities with clinical studies of the Clinical Center.

(c) Supervision over personnel, maintenance, travel, and correspondence.

(d) Requisitioning and supervision of equipment and supplies.

(e) Preparation of reports, budget estimates, and exhibits and other public relations materials.

(f) Maintenance of reference library, reprint files, card catalogues, and specimen collections.

(g) Consultatory services to individuals, commercial organizations, nongovernmental organizations, and Government agencies including liaison activities with other branches of the Service.

(h) Reviewing, editing, and revising scientific and technical reports and manuscripts.

9. (continued)

Research projects are reviewed quarterly. If necessary, they are discontinued or revised in accordance with current developments and needs. During the past calendar year, several changes have been made in the research program of the Laboratory and some activities directed into new channels. Because of extensive revamping of the program last year, this year's alterations have been less marked. Continued emphasis is being placed on the more fundamental aspects of problems in tropical medicine, such as the metabolic requirements of parasites and the chemistry of their metabolism, the physiology of parasites in relation to their disease-producing proclivities, the mechanism of drug action on parasites and the inhibiting effect of antimetabolites, the employment of the newer techniques of fluorescent and radioactive isotope tagging in basic studies in immunology, virus-vector-tissue relationships, and the carriage of viruses by parasites.

New research projects were started on the possible carcinogenetic effect of the larval tapeworm, _Cysticercus fasciolaris_, in rodents to supplement similar studies involving _Schistosoma haematobium_, the cultivation of arthropod tissues _in vitro_ in the hope of using such tissues for the growth of viruses, the mechanism of drug action on _Toxoplasma gondii_, and the deleterious action of that and other protozoan parasites on cancer cells.

Budget Data Sheet

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12. BUDGET ACTIVITY

RESEARCH

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATION PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957.

None

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PHS (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS) IDENTIFY SUCH RESEARCH.

None

15.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

Schmidt, L. H. and Coatney, G. Robert: Review of investigations in malaria chemotherapy (U.S.A.) 1946 to 1954. Am. J. Trop. Med. & Hyg. 4: 208-216, March, 1955.

Wright, W. H.: The laboratory approach to problems in amebiasis. Am. J. Gastroenterology, 23: 303-306, April, 1955.

Phillips, B. P.; Wolfe, P. A.; Rees, C. W.; Gordon, H. A.; Wright, W. H.; and Reyniers, J. A. Studies on the ameba-bacteria relationship in amebiasis. Comparative results of the intracecal inoculation of germfree, monocontaminated, and conventional guinea pigs with Entamoeba histolytica. Am. J. Trop. Med. & Hyg. 4: 675-692, July, 1955.

Coatney, G. Robert: Estado actual de las drogas antipaludicas cloroquina, pirimetamina (Daraprim) y primaquina. Bol. Ofic. san. panam. 39: 175-182, August, 1955

Nadel, Eli M.; Greenberg, Joseph; Jay, George E.; and Coatney, G. Robert: Backcross studies on the genetics of resistance to malaria in mice. Genetics, 40: 620-626, September, 1955.

Shimkin, M. B.; Mustacchi, P. O.; Cram, E. B.; and Wright, W. H.: Lack of carcinogenicity of lyophilized Schistosoma in mice. J. Nat. Cancer Inst., 16: 471-474, October, 1955.

Wright, W. H.: Parasitism in Southeastern United States. Current status of parasitic diseases. Pub. Health Rpts., 70: 966-975, October, 1955.

Brady, Frederick J. and Wright, W. H.: Parasitic diseases of the liver. In Practical Management of Disorders of the Liver, Pancreas, and Biliary Tract by John Russell Twiss and Elliott Oppenheim. Lea and Febiger, Philadelphia, pp. 385-389, 1955.

Coatney, G. Robert: The present status of the antimalarial drugs chloroquine, pyrimethamine (Daraprim) and primaquine. Industry and Tropical Health, Second Conference, 1955.

17. HONORS OR AWARDS FOR THE PROJECT

Wright, Willard H.

Reappointed as Visiting Lecturer on Tropical Public Health, Harvard School of Public Health.

Reelected a Director of the American Foundation for Tropical Medicine Inc. and of the Liberian Institute of the American Foundation for Tropical Medicine Inc.

Elected as one of the Directors for the term 1953-1956 and reelected as a member of the Executive Committee for 1954-1955 of the Gorgas Memorial Institute of Tropical and Preventive Medicine, Incorporated.

Elected Vice President of the American Society of Tropical Medicine and Hygiene.

Appointed to the Committee on Development and Needs of the American Society of Tropical Medicine and Hygiene.

Reappointed Professorial Lecturer in Medical Zoology, School of Medicine, The George Washington University.

Coatney, G. Robert

Appointed Visiting Lecturer in Tropical Public Health, Harvard School of Public Health

Appointed Visiting Professor in the Department of Preventive Medicine and Public Health, Howard University, School of Medicine.

Reappointed Visiting Lecturer on Malaria, Georgetown University, School of Medicine.

Reappointed Chairman of the Editorial Board of the American Journal of Tropical Medicine and Hygiene.

Reappointed to the Panel on Malaria, Division of Medical Science, National Research Council.

Project Description Sheet

1. National Microbiological Institute
INSTITUTE

2. Laboratory of Tropical Diseases
LABORATORY OR BRANCH

3. Office of the Chief
SECTION

4. Rio de Janeiro, Brazil; Cairo, Egypt; Panama, R. de P.
LOCATION

5. NMI - 56
SERIAL NO.

6. Foreign Field Stations
PROJECT TITLE

7. Dr. Willard H. Wright
PRINCIPAL INVESTIGATOR

8. Dr. G. R. Coatney; Dr. E. G. Berry; Dr. C. G. Dobrovolny; Dr. L. Rosen; and Mr. Clinton S. Smith.
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

At the request of U. S. Government agencies or other agencies, the Laboratory of Tropical Diseases is engaged in cooperative research projects in various foreign areas as indicated below. On some of these projects, the Laboratory furnishes the personnel while salaries and other costs are assumed by the cooperating agency or agencies. In other cases, the Laboratory maintains the salary of the individual and the other agency or agencies assume all other costs, including travel. These foreign research projects are of advantage in a number of ways. They furnish opportunities for contacts with foreign investigators; they provide facilities for Laboratory personnel to gain valuable experience with tropical diseases in an endemic area; and more importantly they offer the means for the development and exploitation in the field of the results of basic laboratory investigations.

Certain changes in foreign projects took place during the year. Dr. Leon Rosen returned from duty at the Gorgas Memorial Laboratory, Panama, Republic of Panama in October and was reassigned to the Laboratory of Infectious Diseases. Dr. Elmer Berry left Cairo, Egypt in late December. He had been on assignment as director of the pilot project on snail control being carried out jointly between the International Cooperation Administration, the Egyptian Ministry of Health and this Laboratory. Dr. Charles G. Dobrovolny was reassigned to the Rio de Janeiro Office of the Pan American Sanitary Bureau, to serve as expert consultant on the Schistosomiasis Control Program of the Brazilian Government.

Descriptions of various field projects follow.

9. (continued)

Project Subtitle:

Control of Snail Intermediate Hosts of the Human Schistosomes in Egypt.

Objectives:

To carry out pilot studies on chemical control of snail intermediate hosts to reduce schistosome infection in man.

Methods Employed:

This project was instituted in July, 1954 for the purpose of evaluating the efficacy of sodium pentachlorophenate, a new molluscacide, for the control of the molluscan intermediate hosts of the human schistosomes in Egypt. The area selected was that of Waraq el-Arab, some 10 kilometers northwest of Cairo, and comprising approximately 40 square miles. Irrigation water is supplied from the Nile through 184 kilometers of canals and drains. After initial snail surveys and examinations to determine the incidence of schistosome infection of school children in the area, the first application of sodium pentachlorophenate was made on October 20, 1954. Two and one-half tons of the chemical were required to provide a residual of 10 ppm in the main intake, the Sawahil Canal, which is 24 kilometers in length. An additional 3½ tons were required to treat the remaining 160 kilometers of secondary and tertiary canals and drains. Some of the heavy vegetation was removed from drains by means of a steel basket operated from a tractor. Certain areas in which it was not feasible to remove vegetation were sprayed with a solution of the chemical by means of a fire hose. Following the initial application, all snails were destroyed within the area. The area was retreated on March 17 and 18, and October 6, 1955, because of snails and snail eggs washed in from the Nile.

The original project was so successful that the Egyptian Government requested treatment with sodium pentachlorophenate of the Giza Canal System comprising over 8,000 kilometers of canals and drains. A total of 21,588 pounds of the chemical was inserted into the Giza System. All snails within the area were destroyed with the exception of a few at localized points in which farmers had dammed the secondary and tertiary canals.

Major Findings:

Sodium pentachlorophenate has proved to be an extremely useful chemical for snail destruction under conditions in Egypt. The stability of the chemical in the highly turbid Nile water far exceeded expectations, since it maintained its original residual of 10 ppm. after being carried up to 30 kilometers and was still

9. (continued)

present in certain drains in this concentration six weeks after application. The incidence of Schistosoma haematobium in school children of 6 to 10 years in the Waraq el-Arab area was decreased 8 percent during the year. There has been no transmission of the disease within the area since the first application of the chemical.

Significance to Microbiological Research:

Schistosomiasis constitutes the major public health problem in Egypt. Egyptian authorities estimate that it is responsible for a loss of 33 percent in the productivity of the country. Prior to the demonstration discussed above, efforts to control the disease by various means over a period of years had not resulted in any great progress. More efficient methods are needed for treatment of infected individuals and for the destruction of the snail intermediate hosts. It is believed that the present pilot experiment has demonstrated the value of this new molluscacide in control of the disease.

Proposed Course of Project:

The area will be maintained under close surveillance and additional chemical applied, if and when needed. Since the project has fulfilled its original purpose of demonstrating the value of NaPCP under Egyptian conditions, the Egyptian Government has agreed to assume full control as of July 1, 1956 and will continue the project as an evaluation one with advice from LTD personnel.

Project Subtitle:

Studies on the chemical control of molluscan intermediate hosts of schistosomiasis in Brazil.

Objectives:

To conduct field trials with newer molluscacides for the elimination of molluscan intermediate hosts of human schistosomiasis and to act as Special Consultant for the overall anti-schistosomiasis program.

Methods Employed:

Surveys were carried out by the National Malaria Service during the early part of the year and control projects were then inaugurated in the cities of Araxa in August and in Barreiro in September; both these cities are in the State of Minas Gerais. Each site offered areas of flowing water and therefore the main treatment was by NaPCP balls applied at the rate of 15 ppm for 10 to 12 hours. A motor powered pump was used to spray NaPCP in solution on the swamps, lakes, and banks of streams. Treatment of the large João Pessoa area in the State of Paraiba was carried out in late December using NaPCP in solution.

9. (continued)

Major Findings:

Sodium pentachlorophenate is an extremely useful compound for the killing of snails in Brazil. Selected areas must be carefully surveyed and properly prepared if the killing agent is to be highly effective. This was made plainly evident by the finding of living snails in all but one of the streams in Barreiro in November following treatment during the dry season in August. The fact that vector snails can live out of water and often in very dry localities for many months makes their control and/or elimination difficult and expensive, because the killing agents now available are not effective when the snails are on dry ground and retracted within the shell.

As a result of the aid and information furnished by the project, Brazil has now undertaken a large scale control program using NaPCP supplemented by treatment of infected people, education in principles of sanitation, and by providing additional sanitary facilities.

Significance to Microbiological Research:

Schistosomiasis has been uncontrolled in Brazil prior to this project because no effective control methods were known. As of now, however, effective snail control is possible and at a reasonable cost.

Proposed Course of Project:

Our officer will continue to serve as expert consultant during 1956 and will conduct small scale experimental work to meet local problems.

Project Subtitle:

Studies on Dengue and Related Viruses.

Objective:

To obtain additional information on the epidemiology of dengue in Panama and to determine if virus reservoirs exist in that area; to determine if dengue vectors, other than Aedes aegypti, exist in Panama; and to secure information concerning the prevalence of other related virus infections.

Methods Employed:

Patients with fevers of unknown origin were studied in the Gorgas Memorial Hospital and other hospitals in the Canal Zone and in Panama City. Acute convalescent sera were obtained and tested serologically for dengue and other viruses; serological surveys, involving more than 1,600 samples, were made of populations living under the various environmental conditions in Panama. A similar survey was made among indigenous primates.

9. (continued)

Haemagglutination-inhibition (HI) tests and neutralization tests in suckling mice were employed to detect the presence of dengue antibodies. Seven local species of wild primates were tested in the laboratory for susceptibility to the two immunological types of dengue virus.

Major Findings:

None of the fevers of unknown origin proved to be dengue. Evidence of previous infection with type 2 dengue virus confirmed by neutralization tests, was found among individuals born before mid-1942; prevalence of persons with dengue antibodies appeared higher in urban than rural areas. No evidence of natural dengue infection was found among local wild primates, although all the species (7) could be infected with both types of dengue virus in the laboratory. At least one species exhibited a definite viremia. Studies on recently arrived military personnel, who gave a history of vaccination against yellow fever (y.f.), demonstrated that infection with the 17 D type y.f. virus rarely if ever gives rise to HI antibodies against dengue viruses although practically all had neutralizing antibodies against y.f. virus.

Studies of cross immunity, in monkeys, between virulent y.f., 17 D y.f. and types 1 and 2 dengue indicated that both strains of y.f. virus and the dengue viruses are closely related serologically. Further, it appeared that type 2 dengue is more closely related to y.f. than is type 1.

Significance to Microbiological Research:

Prior to this study it was definitely felt in some quarters that dengue was endemic in Panama. The results of the present study indicate, however, that dengue infection is not now present in Panama and appears not to have been after the extensive epidemic in 1941-42. The dengue infection present during the epidemic appears now to have been of type 2 only and was transmitted mainly if not exclusively, by Aedes aegypti. This mosquito is no longer present in Panama and therefore the dengue viruses could not be transmitted unless unknown vectors exist.

Proposed Course of Project:

The project was discontinued in Panama September 30, 1955. Laboratory work is being continued at the NIH and will not be completed until about March 31, 1956.

Budget Data Sheet

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12. BUDGET ACTIVITY:

RESEARCH

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957.

Project Subtitle: Control of Snail Intermediate Hosts of the Human Schistosomes in Egypt. Department of State, Foreign Operations Administration provides funds for salaries, travel, and equipment; Ministry of Health of Egypt provides technical personnel.

Project Subtitle: Studies on the Chemical Control of the Molluscan Intermediate Hosts of Schistosomiasis in Brazil. Pan American Sanitary Bureau provides funds for travel; Ministry of Education and Health of Brazil provides facilities and technical assistance.

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PHS (WITHOUT INTERCHANGE OR PERSONNEL, FACILITIES OR FUNDS) IDENTIFY SUCH RESEARCH.

None.

R. P. C. - 3 Analysis of NIH Program Activities Serial No. NMI -56
December 19553

Honors, Awards, and Publications Sheet

15.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

Burch, Thomas A.: Proyecto piloto de una campaña de tratamiento médico de la oncocercosis, basado en la administración de suramina sódica (U.S.P. XIII) Parte I. Encuesta pretratamiento. Bol. Oficina San. Panamer., 38, 20-25, January 1955.

Burch, Thomas A. and Greenville, Henry J.: Filariasis in Liberia. Am. J. Trop. Med. & Hyg., 4, 47-51, January 1955.

Dalmat, Herbert T.: Onchocerciasis, a blinding disease endemic in the Western Hemisphere. Nursing Outlook, 3, 23-25, January 1955.

Burch, Thomas, A.; Aguilar, Germán G.; Barrera, Manuel; and Dalmat, Herbert T.: Proyecto piloto de una campaña de tratamiento médico de la oncocercosis, basado en la administración de suramina sódica (U.S.P. XIII) Parte II. Tratamiento y observación subsiguiente. Bol. Oficina San. Panamer., 38, 141-147, February 1955.

Burch, Thomas A.: Treatment of wuchereriasis and onchocerciasis with suramin sodium. Am. J. Trop. Med. & Hyg., 4, 332-333, March 1955.

Dalmat, Herbert T.: Cutaneous myiasis of the scalp due to *Dermatobia hominis* (Linnaeus Jr.) (Diptera; Cuterebridae). Am. J. Trop. Med & Hyg., 4,334-335, March 1955.

Rosen, Leon: Observations on the epidemiology of human filariasis in French Oceania. Am. J. Hyg., 61, 219-248, March 1955.

Dalmat, Herbert T.: The black flies (Diptera, Simuliidae) of Guatemala and their role as vectors of onchocerciasis. Smithsonian Misc. Collections, 125, (Publ. 4173), 425 pp., 43 plates, 13 text fig., 15 maps, 36 tables, April 5, 1955.

Lea, Arden O., Jr.: Two items of equipment useful in black fly control. J. Econ. Ent., 48, 202-203, April 1955.

Olivier, Louis; The natural history and control of snails that transmit the schistosomes of man. Am. J. Trop. Med. & Hyg., 4, 415-423, May 1955.

16. (continued)

Lea, Arden O., Jr. and Dalmat, Herbert T.: Field studies on larval control of black flies in Guatemala. J. Econ. Ent., 48, 274-278, June 1955.

Dobrovolny, Charles G. and Dobbin, James E., Jr.: Initial field trials with some molluscacides in Brazil. Rev. Brasileira Malariol. e Doenças Tropicais, Publ. Avulsas No. 1, 1-19, July 1955.

Lea, Arden O., Jr. and Dalmat, Herbert T.: A pilot study of area larval control of black flies in Guatemala. J. Econ. Entomol., 48, 378-383, August 1955.

Burch, Thomas A.; Qualls, Donald M.; and Greenville, Henry J.: Onchocerciasis in Liberia. Am. J. Trop. Med. & Hyg., 4, 923-929, September 1955.

In press

Gelfand, Henry M.: Anopheles gambiae Giles and Anopheles melas Theobald in a coastal area of Liberia, West Africa. Trans. Roy. Soc. Trop. Med. & Hyg.

Dobrovolny, Charles G.: A summary of field trials with molluscacides in Brazil. National Malaria Service of Brazil.

Dalmat, Herbert T.: The authorship of Onchocerca volvulus (Leuckart, 1893) Railliet and Henry, 1910. Bulletin on Zoological Nomenclature.

Dalmat, Herbert T.: Is Simulium dinellii (Joan, 1912) (Diptera, Simuliidae) present in Guatemala? Ann. Entomol. Soc. Am.

Olivier, Louis: The location of the schistosome vectors, Australorbis glabratus and Tropicorbis centimetralis, on and in the soil on dry natural habitats. J. Parasitol.

Olivier, Louis and Barbosa, Frederico Simões: Seasonal studies on Australorbis glabratus Say from two localities in Eastern Pernambuco, Brazil. Publicações Avulsas, Inst. Aggeu Magalhaes.

Olivier, Louis and Barbosa, Frederico Simões: Seasonal studies on Tropicorbis centimetralis in Northeastern Brazil. Publicações Avulsas, Inst. Aggeu Magalhaes.

16. (continued)

Olivier, Louis and Schneiderman, Marvin: A method for estimating the density of aquatic snail populations. Exper. Parasitol.

Olivier, Louis: Observations on vectors of schistosomiasis mansoni kept out of water in the laboratory. I. J. Parasitol.

Olivier, Louis and Barbosa, Frederico Simões: Observations on vectors of schistosomiasis mansoni kept out of water in the laboratory. II. J. Parasitol.

17. HONORS OR AWARDS FOR THE PROJECT

No honors.

Analysis of NIH Program Activities

Project Description Sheet

1. National Microbiological Institute
INSTITUTE

2. Laboratory of Tropical Diseases
LABORATORY OR BRANCH

3. Epidemiology
SECTION

4. Columbia, S. C. and Memphis, Tenn.
LOCATION

5. NMI - 57
SERIAL NO.

6. Epidemiology of Parasitic Diseases
PROJECT TITLE

7. Dr. Martin D. Young
PRINCIPAL INVESTIGATOR

8. Dr. D. E. Eyles, Dr. C. L. Gibson, Dr. R. W. Burgess, Dr. G. B. Wolcott, Dr. G. M. Jeffery.
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Project Subtitle:

Virus-parasite Relationships.

Objectives:

To determine experimentally if parasites might be associated with some of the virus diseases of man or animals as vectors or in some other capacity. If experimental evidence establishes such relationships between parasites and viruses, further objectives are to study such relationships under natural conditions and to investigate known virus infections and known populations to determine existing relationships. This includes the study of possible intestinal viruses among institutionalized mental patients. To investigate the possibility that certain parasites may themselves be afflicted with harmful virus infections. To investigate the effects of parasitic infections upon virus infections and vice versa. To investigate possible relationships between various arthropods and viruses, other than as biological vectors, including the maintenance of infective virus through immature stages of insects following acquisition by ingestion or other means and investigation of viruses of arthropods and their medical importance.

Methods Employed:

Small animals and their common parasites are used in attempts at transmission of viruses. Viruses, such as LCM, are maintained in small animals by serial transmission, providing infective virus material for experimental work. Tissue culture or small animals are used for detection of virus infection carried by parasites in virus-infected animals, for virus detection in parasites

9. (continued)

suspected as harboring viruses in nature, and for detection of virus infection in arthropods. Incidence of intestinal viruses and their possible association with parasites in institutionalized mental patients is studied with animal inoculation, tissue culture, and other means.

Major Findings:

Most of the experimental work in this project has been in progress for less than a year. In one attempt to confirm results previously reported by others, it was possible to show transmission of LCM virus with Trichinella spiralis in mice, such transmission probably, but not certainly, within the parasite. Progress has been made in refinement of techniques of isolation of parasites without adherent virus and in virus-free suspending fluid.

Conclusive results have been obtained in the mechanical introduction into rabbits of papilloma virus by the penetration of filariform larvae of Nippostrongylus muris. Positive, but less conclusive, results have been obtained using the same helminth with the rabbit fibroma virus.

In preliminary experiments, it has not been possible to demonstrate transmission of virus (LCM or Coxsackie) by common helminth parasites of the mouse.

The results of preliminary experiments are suggestive that larvae of Anopheles quadrimaculatus are capable of ingesting LCM virus with food and maintaining the virus in infective condition through the larval and into the adult stages.

Significance to Microbiological Research:

Many problems associated with the transmission of viruses in human and animal populations remain unsolved. New viruses in mammals and arthropods are continually being discovered. There are suggestions in the literature and theorectical possibilities that parasites may act as reservoirs and agents of transmission for viruses of importance to man. Elucidation of associations of viruses and parasites, both experimental and natural, will do much toward bringing about a more complete understanding of the epidemiology of diseases produced by such organisms alone or in combination.

9. (continued)

Proposed Course of Project:

During the ensuing year all projects will be continued. Because all of the experimental work to date has been preliminary in nature and has indicated promise of positive results along the same lines, the general course of the project will be little changed. All of the projects will be expanded to the extent that different virus-parasite-host combinations will be used when advantageous. It is also hoped that the work may be extended to the available human populations, using the viruses and parasites which they afford.

Project Subtitle:

Cytological Studies of Parasitic Protozoa.

Objectives:

To study chromosome behavior, including movement other than mitotic movement, chromosome cycles, and the point in the life cycle at which meiosis occurs, in order to lay a basis for possible genetic studies.

Methods Employed:

The nuclei of malaria parasites and related organisms are studied, fixed and stained and in living condition under bright field and phase contrast microscopes. The parasites are studied at all possible stages of their life cycles, under normal and experimental conditions.

Major Findings:

Mitosis has been demonstrated in *Plasmodium vivax* and chromosome numbers and morphology established for all four species of human malaria. Further study was done on *P. berghei* and a manuscript is being prepared to describe the chromosome pattern of certain rodent, avian, simian, and saurian malarial parasites. The chromosome patterns fit the supposed relationships among these parasites. It is felt that the survey of chromosome numbers and patterns in *Plasmodium* has been extended as far as would be profitable, and this portion of the project is considered completed.

Significance to Microbiological Research:

There is little basic knowledge of the fundamental nuclear structure of most of the human parasites. Knowledge of the nuclear structure and behavior will aid in many fields, such as taxonomy, drug fastness, virulence, and will make possible an understanding of the inheritance of these parasites.

9. (continued)

Proposed Course of Project:

During the next year the major emphasis will be on locating the point in the life cycle of Plasmodium at which meiosis occurs and to describe meiosis. Other observations will be made along with this.

Project Subtitle:

Epidemiology and Chemotherapy of Intestinal Parasites in an Institutional Population.

Objectives:

To investigate factors governing the spread of intestinal parasitism in an institutional population, to investigate means for elimination of parasite infections and spread, and to find better drugs to treat infections of hookworm, Strongyloides and Trichuris, which constitute serious health problems in certain types of mental patients. Whenever possible, knowledge gained from work within the institution is applied to more normal populations in an effort to provide answers to some of the existing epidemiological problems.

Methods Employed:

Many epidemiological approaches are used to elucidate differential transmission of various parasites, including examination of several types of institutionalized patients, examination of premises for contamination, and other methods. Included is the trial of promising compounds against various helminth and protozoan intestinal parasite infections.

Major Findings:

Examinations of a closely controlled population in a single building have yielded interesting results in fluctuations of parasitic infections. The overall incidences of parasites in the group have remained quite stable, with several exceptions. The protozoan parasites have continued to be transmitted from patient to patient with considerable frequency. In spite of apparent lack of new cases of hookworm and Trichuris, the rates of infection have remained high, indicating little loss of worms over the period of observation. In contrast, another worm, i.e., Strongyloides, showed a progressive decrease in prevalence. Contamination in the building under observation (a new building at the outset of the experiment) has been found intermittently, but has not built up to any overwhelming level in about fifteen months.

9. (continued)

Investigation of an outbreak of diarrheal disorders in mental defective patients revealed four cases of Isospora infections, apparently the first report of this parasite in the State. Evidence indicated a direct association of the parasite with the diarrheal episodes.

Many of the parasites which are found in highest incidence in the institution are those for which there is no satisfactory treatment. A number of compounds have been tested with some encouraging results. An optimum regimen for using piperazine citrate against Ascaris has been indicated. A new drug, Vermiplex (R), is showing promise against hookworm. Puromycin has been shown to eradicate asymptomatic infections of Entamoeba histolytica at safe dosages and also appears promising against symptomatic infections. It exhibits the unusual property of eliminating all of the common protozoa from the gut.

Where material is available, studies have been made of life histories of some individual parasites with the objective of more efficient control or treatment in human populations. This applies especially to the rarer parasites, such as Isospora belli, which occasionally are available in the group.

Significance to Microbiological Research:

To understand more clearly the factors affecting the spread of parasitism in normal populations, much can be learned utilizing the controlled population within an institution. The higher incidence of parasitism in the institutional group makes it an ideal group for study of these parasites and their epidemiology. Much should be learned about the relationship of parasite spread to housing, age, length of residence, and other variables. It seems evident that mental hospitals are important reservoirs of parasitic infections in this country. The development of drugs and other methods to reduce this parasitism is important for the health of the mental patient as well as for use in more normal situations.

Proposed Course of Project:

The epidemiological study of parasites in the institutional population followed over a fifteen-month period will be continued for the current year, with emphasis on the rate of tansmission of various parasites, the possible increased rate of loss of those parasites which have a low transmission rate, increased contamination in the building, et cetera. Trials of new antiparasitic drugs will be continued; use of puromycin in repeated small dosages as a prophylactic against intestinal protozoa in a highly infected institutionalized population will also be tested. Biological studies of parasites whenever unusual material becomes available will be done to throw light on parasite epidemiology.

9. (Continued)

Project Subtitle:

Cytogenetics and Species Relations of Insect Vectors of Disease.

Objectives:

Correlation between chromosomal patterns and inheritance of morphological and physiological characters in species, populations, hybrids, and selected breeding lines of mosquitoes and other arthropods. Determine validity of and relationships between species.

Methods Employed:

Rear and study characters of species, variants of species, hybrids, and selected strains. Make cytological studies and chromosome maps of as many as possible for study and comparison.

Major Findings:

Observations mostly on *Anopheles quadrimaculatus*, indicate that chromosome number and morphology are in line with previous work on other anophelines, but that there are significant chromosomal variations within populations and between populations which will be useful in analysis within species and between species. The sex chromosomes (chromosome 1) has been identified at meiosis in the male and in limb-bud and giant salivary gland chromosomes in males and females. It is the shortest chromosome, attached to the nucleolus, and partially heterochromatic. It is haploid in males and diploid in females. However, at male meiosis an apparent Y chromosome is present. This may be similar to the condition in *Drosophila*, but there are alternative explanations. The numbers of branches on certain hairs have been influenced by selection, but such selected strains exhibit high mortality. Hybrid individuals between anopheline species have been obtained but did not reproduce.

Significance to Microbiological Research:

Ability of arthropods to transmit disease and susceptibility to insecticides as well as the physiological and morphological characters may depend upon specific characters, inheritance of which may be demonstrated by a combination of breeding, chromosomal, and taxonomic studies.

9. (continued)

Proposed Course of Project:

Continue hybridization of species with attempts to establish hybrid or backcross colonies. Chromosome studies will be related to this work. Conduct experiments in inheritance and analyze the results for (a) type of inheritance, and (b) possible relation (linkage) with ability to transmit disease, virus or otherwise, and with resistance to insecticides.

Project Subtitle:

Malaria Studies.

Objectives:

To add to the biological knowledge of malaria by limited observations on induced malaria and experimental studies. To serve as probably the only source in the nation for established strains of malaria for use on a nationwide basis for the therapy of neurosyphilis, nephrosis, Parkinsonism, and other conditions and for experimental malaria studies.

Methods Employed:

Malaria is induced in neurosyphilitic patients and in volunteers under normal and experimental conditions. During the course of the infection, pertinent biological information is obtained. Malaria is being furnished to several institutions to treat nephrosis and for experimental work.

Major Findings:

Additional observations have shown that the parasites can be safely preserved at -70° C. for periods longer than one year which greatly facilitates the maintenance of documented species and strains.

Contrary to prevailing accepted opinion, *Plasmodium falciparum* occasionally relapsed after both single and multiple doses of chloroquine.

Chronic infections of *P. falciparum*, a year following initial infection, and with extremely low gametocyte densities are capable of mosquito transmission.

9. (continued)

A study of the Chesson strain of P. vivax has shown that all sporozoite-induced cases followed sufficiently long were found to relapse after treatment. First relapses occurred after longer periods of latency, ranging from two to twelve months, than previously had been reported, undoubtedly, due to the more extensive clinical course experienced by our patient (neurosyphilitics) than in the subjects of previous reports (volunteers).

Comprehensive data gathered over a twenty-five-year experience conclusively demonstrated that the American Negro generally is immune to P. vivax malaria. This could not be overcome by massive infective inoculations and appears to be a natural immunity.

Significance to Microbiological Research:

The transmission potential of chronic falciparum malaria and the relapses following chloroquine therapy are of epidemiological importance in endemic zones and in control planning. In Chesson vivax malaria, our experience more closely simulates natural field conditions than some other experimental procedures and indicates that relapses, often late, must be expected after chloroquine therapy and that such relapsing malaria can be transmitted.

The continued maintenance of established strains of malaria for the therapy of neurosyphilis, nephrosis, and other conditions, is of considerable importance to those dependent upon this procedure for adequate and often last-resort therapy.

The immunity of Negroes to vivax malaria is a striking demonstration of the selective resistance of a human race to a single species of parasite, while remaining susceptible to closely related species. So far as we know, this is the only authenticated instance in the field of human parasitology and has important implications in the general field of host-parasite relationships.

Proposed Course of Project:

It will operate along the presently curtailed lines with the principal activity being that as a center for furnishing inocula for the treating of neurosyphilis, nephrosis, and other diseases.

Project Subtitle:

Chemotherapy of toxoplasmosis.

9. (continued)

Objectives:

The primary objective of this project is to evaluate the effect of known antitoxoplasmic agents in man and in laboratory animals and to find new drugs, particularly drugs active against the chronic stages of the parasite. Secondarily, the objective is to study the fundamental physiologic mechanisms of the parasite as related to chemotherapy.

Methods Employed:

The effect of drugs of known activity such as pyrimethamine and sulfonamides is being studied in man in cooperation with local ophthalmologists and internists. Correlated studies are made in laboratory animals and domestic animals. Tissue culture techniques are used for studies of the effect of metabolites in reversing drug action and the effect of antimetabolites on the growth of the parasite.

Major Findings:

Studies in cooperation with several local ophthalmologists have resulted in the treatment of a number of cases of presumptive toxoplasmic uveitis with apparent successful results in a number of cases. Continued studies in animals have shown that pyrimethamine acts synergistically with sulfonamides other than sulfadiazine and also with some sulfones. A comparison of the degree of interaction indicates differences, and these are being studied. In vitro experiments have shown that pyrimethamine and sulfadiazine are active against Toxoplasma infections in HeLa cultures. Pyrimethamine and sulfonamides have been found active in canine toxoplasmosis, but further evaluation is needed. Other experiments have resulted in the discovery of a number of additional drugs with some antitoxoplasmic activity, but the significance of these findings has not yet been determined.

Significance to Microbiological Research:

Additional evidence has accumulated that pyrimethamine and sulfonamides may have an effect against acute toxoplasmosis in man, and much evidence is accumulating indicating important effect against toxoplasmic uveitis and chorioretinitis. In addition to the practical value of the findings in this project, the system is well adapted to the study of fundamental mechanisms of drug action; for instance, evidence is accumulating that pyrimethamine and sulfonamides act by sequential blocking of the metabolic pathway involving p-amino-benzoic acid, folic and folinic acid thus explaining the strong synergism. Such information may be applicable to other organisms also.

9. (continued)

Proposed Course of Project:

Further evaluation of the effect of drugs in man will be carried out to the greatest extent possible. Experiments in animals and in tissue culture will be continued with particular emphasis on studies of the fundamental mode of action of the drugs. Attempts will be made to find other compounds which will block the p-aminobenzoic acid, folic acid, folinic acid sequence at other loci with the optomistic objective of imposing a third sequential block and thus improving the antitoxoplasmic effect. Antipurines in particular will be studied. The results of these experiments will be compared with the effect of similar metabolites on other organisms, and the use of other organisms experimentally will be considered. The effect of metabolites and antimetabolites, other than those previously mentioned, will be explored in order to learn more concerning the physiology of the parasite, and perhaps obtain information allowing a more logical approach to the problem of chemotherapy. Experiments are also planned to find to what extent the toxicity of pyrimethamine and sulfonamides can be alleviated by vitamin use without interference with the anti-parasitic action.

Project Subtitle:

Epidemiology of toxoplasmosis.

Objectives:

To determine the mode of transmission, factors influencing the spread of this infection, and to explore possible relationships to mental diseases.

Methods Employed:

General epidemiological approaches are used to obtain information on various items such as age, sex, race, habitat, occupation, mental condition, housing, and other pertinent factors in relation to serological response of various population groups to the dye test for Toxoplasma antibodies. Parasitological and serological studies are done to determine to what extent wild or domestic animals may serve as a reservoir of human infection. Tests are being run to determine whether the infection is more prevalent or if the infection spreads more rapidly in a mental institution than in the normal population. Any suggestive outbreak in a cooperating mental institution will be investigated epidemiologically.

Major Findings:

Comparative study of a rural Negro population near Memphis and an urban Negro population within the City of Memphis gave serological results which suggested that human infection with Toxoplasma

9. (continued)

is equally common in both environments, the prevalence rate being at least 14 per cent in the second decade of life. No difference between the sexes has been detected with regard to the prevalence of antibodies, which become increasingly frequent with increasing age, reaching a rate of 53 per cent at age 60 and over. Preliminary studies on an urban Caucasian population suggest that comparable prevalence rates will be found in both races. Incomplete studies on blood sera from Guatemala, Central America, suggest that toxoplasmosis is much more frequent in that country than in the United States, and that perhaps it occurs more often in the relatively low tropical coastal regions than in the cooler highlands.

In the investigation of possible reservoir hosts, 16 per cent of dogs tested had significant amounts of antibodies for Toxoplasma, and the parasites were isolated from two of 193 dogs by inoculation of tissues into mice.

Strong confirmative information has been obtained indicating that toxoplasmosis is closely correlated with certain cases of ocular disease.

Significance to Microbiological Research:

Toxoplasmosis is being more widely recognized as a disease entity, and the dye tests show that it probably is of widespread prevalence. The present epidemiological studies should serve to elucidate such important points as how these parasites are transmitted, mode of entrance into the body, the importance of animals as reservoirs of the disease and other factors which need to be known before an intelligent approach can be made to control programs.

The possible relationships of toxoplasmosis to mental conditions are being explored.

Proposed Course of Project:

During the next calendar year serological studies on other population groups will be continued. From epidemiological studies, clues will be sought for methods of determining vectors and reservoirs. Studies will be continued in cooperation with local physicians and professional people toward the end of finding cases of toxoplasmosis and determining the relationship of certain clinical findings to the presence of Toxoplasma organisms. The relationship of mental diseases and of institutional housing of mental patients to toxoplasmosis will be investigated.

9. (continued)

Project Subtitle:

Biological Studies of Toxoplasma gondii.

Objectives:

To investigate parasitologic problems involving Toxoplasma particularly those which arise from epidemiological and chemotherapeutic studies.

Methods Employed:

Toxoplasma infections are studied in various animals which may serve as natural reservoirs of the infection. Those features of the infection which might have bearing on the epidemiology of the disease are particularly observed such as:degree and duration of parasitemia, occurrence of Toxoplasma in body secretions, and the persistence of parasites in the host tissues. Other experiments involve obtaining background basic to studies in chemotherapy and still others involve the measurement of the resistence of various stages of Toxoplasma to the external environment, to radiation, heat, etc.

Major Findings:

Toxoplasma infections in chickens have been found to be of short duration as measured by tissue persistence. Parasitemia is present for about 10 to 12 days and is of low grade although intermittent parasitemia may be found up to a month or more. Adult dogs have been found to survive intravenous inoculations of 10^5 and 10^6 organisms per kilogram of body weight, but the inoculation of 10^7 parasites ordinarily produces severe illness and death. Congenital infections in puppies have been produced by inoculation of the bitch during the last few weeks of pregnancy with inocula of 10^6 and 10^7 organisms but not with smaller inocula. Toxoplasma organisms from mouse peritoneal exudate have a thermal death point of 50° C. for 16 minutes. Approximately 15×10^3 ergs per cm^2 of ultraviolet radiation produce death of exposed Toxoplasma. Quick freezing of Toxoplasma in alcohol-dry ice mixtures was unsuccessful, but slow-freezing in glycerol mixtures allows the preservation of viable organisms for at least six months.

Significance to Microbiological Research:

Since the mode of transmission and the natural reservoir of Toxoplasma from which man acquires infection are unknown studies of infections in animals closely associated with man are necessary. It is believed that knowledge of the characteristics of the infection may point toward possible modes of transmission and may complement epidemiological work on prevalence and on natural hosts.

9. (Continued)

Proposed Course of Project:

Further studies will be made of Toxoplasma infections in animals associated with man, and these will be correlated with studies of the occurrence of natural infections. Experiments on the possible in utero infection of the fetus from chronic Toxoplasma infections are underway. Also projected are studies of possible oncolytic effects of Toxoplasma and other tissue protozoan infections, and this project may be expanded if satisfactory results are obtained. Studies will also continue on the effects of external environmental conditions.

Budget Data Sheet

10.

11.

12. BUDGET ACTIVITY:

RESEARCH

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATION PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957.

The South Carolina State Hospital provides some facilities and also staff cooperation, especially clinical participation, for most of the program.

The Clinton Home for Mental Defectives cooperated by making it possible for us to obtain sera from the patients and by the active participation of one of the staff physicians in drawing blood and evaluating clinical diagnosis.

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PHS (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS) IDENTIFY SUCH RESEARCH.

None

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December 1955

Honors, Awards, and Publications Sheet

15.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

Eyles, D. E. and Jones, F. E.: The prevalence of Endamoeba histolytica and other intestinal parasites in a selected urban area. Am. J. Trop. Med. & Hyg. 3: 988-994.

Jeffery, G. M., Wilcox, A. and Young, M. D.: A comparison of West African and West Pacific strains of Plasmodium ovale. Trans. Roy. Soc. Trop. Med. & Hyg. 49: 168-175.

Rendtorff, R. C. and Holt, C. J.: The experimental transmission of human intestinal protozoan parasites. V. Multiple infections produced with three species of amebae. Am. J. Hyg. 61: 321-325.

Wolcott, G. B.: Chromosomes of the four species of human malaria. J. Heredity XLVI: 53-57.

Young, M. D., Eyles, D. E., Burgess, R. W., and Jeffery, G. M.: Experimental testing of the immunity of Negroes to Plasmodium vivax. J. Parasit. 41: 315-318.

Rendtorff, Robert C.: Toxoplasmosis. The Recorder of the Columbia Medical Society. XIX: 12-21.

Arnold, J., Alving, A. S., Hockwald, R. S., Clayman, C. B., Dern, R. J., Beutler, E., Flanagan, C. L., and Jeffery, G. M.; The antimalarial action of primaquine against blood and tissue stages of falciparum malaria (Panama, P-F-6 Strain). The Jour. Lab. & Clin. Med. 46: 391-397.

Burgess, Robert W.: Experiments in hybridizing Anopheles freeborni Aitken and Anopheles punctipennis (Say). Ann. Entomol. Soc. Am. 48: 229-231.

Young, Martin D.: Parasitism in Southeastern United States: Introduction. Public Health Reports 70: 957.

Jeffery, G. M. and Eyles, D. E.: Infectivity to mosquitoes of Plasmodium falciparum as related to gametocyte density and duration of infection. Am. J. Trop. Med. & Hyg. 4: 781-789.

16. (continued)

Jeffery, G. M. and Rendtorff, R. C.: Preservation on viable malaria sporozoites by low-temperature freezing. Exp. Parasit. 4: 445-454.

Eyles, D. E. and Coleman, N.: An evaluation of the curative effects of pyrimethamine and sulfadiazine, alone and in combination, on experimental toxoplasmosis. Antibiotics and Chemotherapy 10: 520-539.

Eyles, D. E. and Coleman, N.: The effect of sulfadimetine, sulfisoxazole, and sulfapyrazine against mouse toxoplasmosis. Antibiotics and Chemotherapy 10: 525-528.

Eyles, D. E. and Jones, F. E.: The chemotherapeutic effect of pyrimethamine and sulfadiazine on toxoplasmosis of the Norway rat. Antibiotics and Chemotherapy. 5: 731-734.

In Press

Jeffery, G. M.: Relapses with Chesson strain *Plasmodium vivax* following treatment with chloroquine. Am. J. Trop. Med. & Hyg.

Jeffery, G. M.: Blood meal volume in *Anopheles quadrimaculatus* and *A. albimanus*. Exp. Parasit.

Jeffery, G. M., Young, M. D., and Eyles, D. E.: The treatment of *Plasmodium falciparum* with chloroquine, with a note on infectivity to mosquitoes of primaquine- and pyrimethamine-treated cases. Trans. Roy. Soc. Trop. Med. Hyg.

Young, M. D. and Freed, Joe E.: The effect of puromycin against *Entamoeba histolytica* and other intestinal parasites. J. Am. Med. Assn.

Burgess, R. W.: Effect of topically applied DDT on development of *Plasmodium vivax* and *P. falciparum* in *Anopheles quadrimaculatus* Say. Am. J. Trop. Med. & Hyg.

Wilcox, A., Haas, V. A., Hinman, E. H. and Most, H. Laboratory aid in the diagnosis of malaria. Diagnostic procedures and reagents.

Eyles, D. E.: Newer knowledge of the chemotherapy of toxoplasmosis. Ann. N. Y. Acad. Sci.

16. (continued)

Eyles, D. E. and Coleman, N.: Relationship of size of inoculum to time to death in mice infected with Toxoplasma gondii.

Eyles, D. E.: Toxoplasmosis: Summary and challenge. Ann. N. Y. Acad. Sci.

Wettingfeld, R. F., Rowe, J. and Eyles, D. E.: Treatment of toxoplasmosis with pyrimethamine and triple sulfonamide. Ann. Int. Med.

Gibson, C. L., Eyles, D. E., Coleman, N. and Smith, C. S.: Serological response of a rural Negro population to the Sabin-Feldman cytoplasm-modifying test for toxoplasmosis. Am. J. Trop. Med. & Hyg.

17. HONORS OR AWARDS FOR THE PROJECT

Young, Martin D.

President of Sigma Xi Club, University of South Carolina. Member of the American Association for the Advance of Science Council.

Wolcott, Gordon B.

President of LeConte Scientific Society. Executive Committee Member of Association of Southeastern Biologists.

Eyles, Don E. and Gibson, Colvin L.

Received Honorary Appointments as Lecturers in Preventive Medicine, University of Tennessee Medical School.

R.P.C. - 1 Analysis of NIH Program Activities
December 1955

Project Description Sheet

1. National Microbiological Institute
INSTITUTE

2. Laboratory of Tropical Diseases
LABORATORY OR BRANCH

3. Helminthic Diseases
SECTION

4. Bethesda
LOCATION

5. NMI - 58
SERIAL NUMBER

6. Studies of Helminths and Diseases Caused by Them
PROJECT TITLE

7. Dr. Eloise B. Cram
PRINCIPAL INVESTIGATOR

8. Mr. W. B. DeWitt, Dr. W. T. Haskins, Dr. M. F. Jones, Mrs. M. O. Nolan, Dr. L. J. Olivier, and Dr. P. P. Weinstein
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Project Subtitle:

Definition of Nutritional Requirements and Determination of Excretory Products of Parasitic Nematodes.

Objectives:

Development of media suitable for *in vitro* cultivation of nematodes throughout their life cycle, and the development of methods for, and isolation of, the excretory products of such organisms.

Methods employed:

Nematode eggs or larvae are isolated bacteria-free and inoculated into culture media considered capable of supporting growth, and are observed for development and differentiation. Sterile incubates of larval stages are chemically fractionated and analyzed by micro-methods and paper chromatography to identify the excretory products.

Major Findings:

Nippostrongylus muris, a nematode parasite of rats, has been cultivated from the egg to the adult stage *in vitro* under bacteria-free conditions. This represents the first successful cultivation of a nematode parasite of a vertebrate through its entire life cycle. Evidence has also been obtained for vitamin requirements for nematode growth and differentiation, and the need for some water-soluble vitamins has been determined qualitatively and semi-quantitatively. A partially defined medium has been developed for the cultivation of the free-living stages of *N. muris*.

9. (continued)

A qualitative and quantitative determination of the nitrogenous materials excreted by *Trichinella spiralis* larvae has been made. The major constituents were found to be ammonia (35%), amino acids (30%), protein (20%), and aliphatic amines (10%); undetermined minor constituents (5%). A qualitative analysis for the identification of the amino acids in the excreta of the worms has been performed and the following acids have been found present: glutamic, serine, glycine, alanine, tyrosine, valine, methionine, leucine, phenylalanine and proline.

Significance to Microbiological Research:

When the nutritional and physical conditions necessary for development of nematodes, and the identity of excretory products are known, basic *in vitro* studies may explain clinical and pathological effects of nematode infections and enable better diagnosis and treatment.

Proposed Course of Project:

Work will be concerned with the investigation of different tissue and blood fractions to delineate further the growth requirements of nematodes in complex media, and attempts will be made to obtain development in media of known chemical composition. A further study will be made of the nitrogenous materials found in the larval incubates and to gain quantitative information concerning protein and nonprotein excretory products.

Project Subtitle:

Nutritional Aspects of Helminthiasis.

Objectives:

To determine the role played by nutritional factors in the disease processes associated with helminthic infections.

Methods Employed:

Experimental animals maintained on defined diets deficient in one or more factors are exposed to various helminths. Influence of the induced deficiency on the host as well as on the parasite and its relations with the host are evaluated by biological, biochemical, pathological, and physical methods. Special interest is being given to the influence of the deficient state on disease patterns associated with the infection.

Major Findings:

Studies to date have been limited to evaluating the host-parasite relationship of the blood fluke Schistosoma mansoni and mice maintained on a deficient diet known to produce severe liver degeneration. The diet employed was basically deficient in vitamin E, the amino acid cystiene, and factor 3. The major findings in this study include the following:

1. The parasite did not develop to sexual maturity when the host was allowed to become deficient before being exposed to the larval stages of the parasite. If, however, the parasite was introduced before the animal became truly deficient it reached apparent sexual maturity even though its growth was severely stunted.

2. Impairment of natural resistance of the host to infection with S. mansoni was indicated by recovery of a significantly greater number of worms at autopsy from deficient animals than from controls exposed to the same number of cercariae.

3. Animals infected with S. mansoni were found to have impaired ability to digest and absorb dietary fats and proteins.

4. Animals on deficient diet showed altered blood protein patterns characterized by a decrease in total protein content with a lowered A/G ratio. Deficient animals did not show a gamma globulin response to parasitic infection which was marked in animals on control diet.

5. An incidental finding not associated with host-parasite relations may have major significance. Between 90 and 100 per cent of the animals on the deficient diet developed myocardial degeneration and necrosis with up to 50 per cent of the cardiac musculature being destroyed.

Significance to Microbiological Research:

It has become quite clear over a number of years that debilitating helminthiasis occurs very frequently among populations with dietary deficiencies. The disease state of these individuals cannot be properly evaluated until the interaction of these factors has been elucidated. Furthermore, the problem of treatment, prevention and control of helminthic infections may be better approached if these factors are understood.

Proposed Course of Project:

Work will be concerned with investigation of the role played by each of the three factors deficient in the basic diet. Up until now interest has been on the effects of the acute deficiency state. This will be extended by investigating the role of subacute or chronic conditions. Following this, other dietary deficiencies will be studied in relation to helminthic infections.

Project Subtitle:

Possible Cancerogenic Properties of Certain Helminths.

Objectives:

As a cooperative study with NCI, to appraise experimentally the relation reported from clinical observations and statistical surveys as existing between certain parasites and certain malignant growths.

Methods Employed:

Using Schistosoma haematobium:
(a) A large group of hamsters which were exposed to 300 cercariae each are being held to study the effects of long-term active infections with adult, egg-producing worms.
(b) Strain C3H mice were injected with lyophilized worms and/or eggs, to evaluate the possible toxic effects of non-living material; this method was tested and has been suspended, at least for the present.

Major Findings:

(a) One hundred seventy-seven infected hamsters are still living. Forty hamsters have died after having been exposed to infection 162 to 305 days previously. The gross pathology observed includes the following: Bladder wall thickened, vessels enlarged, some containing schistosomes and their eggs; contents of bladder bloody. Hemorrhages in intestines. Abscesses and mesenteric adhesions. Liver cirrhotic. Lungs, heart, kidney with eggs on surface; in one instance a large growth on the right ureter at the distal end of kidney. Report on microscopic pathology not yet received.

(b) At termination of the experiment in which lyophilized schistosome material was injected into mice, there was no evidence of malignancies attributable to the schistosome material.

Significance to Microbiological Research:

Certain members of all three classes of helminths - Nematoda, Cestoda and Trematoda - are found associated with malignant growths. The frequent occurrence of cancer of the urinary bladder correlated with Schistosoma haematobium infection has led physicians in Egypt to ascribe an etiologic role to the parasite. In the Ministry of Health at Accra, Gold Coast, an experimentally-induced infection of the baboon with the local strain of S. haematobium resulted in a malignant growth which greatly thickened the wall of the bladder. Experimental evidence is now sought in small laboratory animals which might prove to be an important tool for cancer research.

Proposed Course of Project

A continuance of long-term study of hamsters infected with the Gold Coast strain of S. haematobium. As individuals die, extensive gross and microscopic studies of the pathology are to be made, for comparison with non-infected controls. A comparable series of hamsters will be infected with the Egyptian strain of the schistosome, if it is procurable and if a stock supply in snails and laboratory animals can be maintained.

Project Subtitle:

An Investigation of Pneumocystis as an Etiologic Agent in Human Cases of Atypical Pneumonia.

Objectives:

A study of the biology of Pneumocystis especially to elucidate its life cycle and method of transmission. This is necessary for prosecution of studies leading toward an investigation of the possible role of Pneumocystis in human cases of atypical interstitial pneumonia.

Methods Employed:

Presence of the organism is determined by microscopical examination of Giemsa-stained smears or impressions of lung tissues. Techniques for isolation and concentration of the pneumocysts are under study. The source of the organism is white rats. Human material so far as proven negative. In order to obtain more material for study and experimentation, attempts are being made to transmit the organism to and produce the disease in laboratory animals by intranasal inoculations and other means. Efforts are continuing to culture the organism in tissues.

Major Findings:

Carriage of the organism by white rats at different age levels has been demonstrated. An incidence of approximately 50 per cent has been observed regularly in young adult white rats. Based on study of parasitological material in the lungs, we have confirmed that reproduction of the pneumocysts is apparently by 3 nuclear divisions, starting with a single nucleus and reaching an 8-nucleated stage, all stages being present in the lungs. Whether or not cytoplasmic partition accompanies nuclear division, or occurs only subsequent to it, is not yet known.

Significance to Microbiological Research:

Aside from the occurrence of isolated cases of pneumocystic pneumonitis in premature infants in northern and central Europe, and South America, very little is known about the organism in its relation to infection and disease in humans. The information gleaned in the laboratory by a study of the organism in animals and by repeated experimentation is fundamental and essential. The broad significance of our findings to date cannot be evaluated at this time. The immediate significance is that we now have a more or less constant source of material for experimental investigation.

Proposed Course of Study:

Efforts will be intensified to demonstrate the organism in humans in this country by a study of postmortem material from premature and newborn infants and to continue laboratory experiments with emphasis on studies of the life cycle and transmission to susceptible laboratory hosts.

Project Subtitle:

Cestode Host-parasite Relationships.

Objectives:

To study the characteristics of infection in laboratory animals with tapeworms, especially *Taenia taeniaformis*; to test how resistance to infection may be enhanced or reduced and to study variations in resistance in various rat and mouse strains; to determine the relation of the larval tapeworm to tumor formation.

Methods Employed:

Various infection techniques including transplantation of the parasites to new hosts and to new locations in the hosts. Various techniques to measure and to transfer resistance. Susceptible and resistant host strains will be sought and development of relatively pure lines of the parasite will be attempted.

Major Finding:

The infections have been established in the laboratory only recently and therefore important new information has not yet been obtained.

Significance to Microbiological Research:

The problems of resistance and immunity to infection are fundamental problems in the field of microbiology and fit in with the renewed emphasis on host reactions to foreign bodies. Worm parasites are excellent tools for the study of immunity because they do not usually multiply in the host and therefore, the number of parasites can be controlled rigidly. Moreover, the parasites can be introduced into the host in limited numbers, removed, and then replaced at a later time; thus there is full control over the amount of antigenic stimulation given to the host.

Because Taenia taeniaformis induces tumors in the rat host and since a virus may be the cause of these tumors, it is thought advisable to carry on additional work on the relationship of the worms to the tumors.

Proposed Course of Study:

The course of the project will be determined largely by the amount of success achieved in standardizing infection, in discovering strains of host with differing susceptibilities, and in revealing indicators of differing resistant states that can be measured quantitatively. Hopefully, it may be possible to study the effect of heredity on host resistance, the effect of hormones on the status of host resistance, the duration of resistance after removal of the parasite, the sites of production of antibody, the location in the resistant host where the parasite is attacked, and the mechanisms by which the host attacks and disposes of the parasite. Observations will be conducted on the tendency of the parasite to produce tumor formation in experimental animals.

Budget Data Sheet

10.

11. Budget Data

12.

BUDGET ACTIVITY

RESEARCH

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATION PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 OR 1957

Project Subtitle: Nutritional Aspects of Helminthiasis. Laboratory of Biochemistry and Nutrition, supplies diets and advice.

Project Subtitle: Possible Cancerogenic Properties of Certain Helminths. Pathologic Anatomy Branch, NCI, providing animal space and care, and making pathological study for the project.

14. None

IF THIS PROJECT RESEMBLES, COMPLEMENTS OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PHS(WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS) IDENTIFY SUCH RESEARCH.

Honors, Awards, and Publications Sheet

15.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

Nolan, M. O. and Bond, Howard W.: Results of laboratory screening tests of chemical compounds for molluscicidal activity. III. Derivatives of abietic acid. Am. J. Trop. Med. & Hyg. 4, 152, January 1955.

Weinstein, Paul P.: The effect of cortisone on the immune response of the white rat to Nippostrongylus muris. Am. J. Trop. Med. & Hyg, 4, 61, January 1955.

DeWitt, William B.: Influence of temperature on penetration of snail hosts by Schistosoma mansoni miracidia. Exptl. Parasitol., 4, 271-276, May 1955.

Olivier, Louis: The natural history and control of the snails that transmit the schistosomes of man. Am. J. Trop. Med. & Hyg., 4, 415, May 1955.

Weinstein, Paul P. and Haskins, Willard T.: Chemical evidence of an excretory function for the so-called excretory system of the filariform larva of Nippostrongylus muris. Exptl. Parasitol., 4, 226, May 1955.

Shimkin, Michael B.; Mustacchi, Piero O.; Cram, Eloise B.; and Wright, Willard H.: Lack of carcinogenicity of lyophilized Schistosoma in mice. J. Nat. Cancer Inst., 16, 471-474, October 1955.

Brooke, M. M., Swartzwelder, C. J., Payne, F. J., Weinstein, P. P.; and Frye, W. W.: An intestinal parasite survey of a Korean prisoner of war camp located on Koje island. In Press: U. S. Armed Forces Med. J.

17. HONORS OR AWARDS FOR THE PROJECT

Dr. Louis J. Olivier was appointed Editor of Tropical Medicine and Hygiene News.

Dr. Paul Weinstein was appointed to the Editorial Board of the Journal of Parasitology.

Dr. Eloise B. Cram was inducted as President of the American Society of Parasitologists.

R. P. C. - 1 Analysis of NIH Program Activities
December 1955

Project Description Sheet

1. National Microbiological Institute
INSTITUTE

2. Laboratory of Tropical Diseases
LABORATORY OR BRANCH

3. Protozoal Diseases
SECTION

4. Bethesda
LOCATION

5. NMI - 59
SERIAL NO.

6. Studies on the Biology and Pathogenesis of Protozoan Parasites of Man
PROJECT TITLE

7. Dr. Charles W. Rees
PRINCIPAL INVESTIGATOR

8. Dr. Leon Jacobs, Dr. H. D. Baernstein, Dr. Thomas Burch, Miss L. V. Reardon, Miss M. L. Melton, Miss M. K. Cook, Mr. B. P. Phillips
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Project Subtitle:

Studies on Amoebiasis.

Objectives:

The long-range objectives of investigations in amoebiasis are: Information on the growth factors of *Endamoeba histolytica* in pure culture as prerequisite for metabolic investigations of the parasite, experiments on infectivity and pathogenicity as a basis for effective chemotherapy, and clarification of prerequisites for immunodiagnosis.

Methods Employed:

The methods include the development, maintenance, and use in experiments as indicated above, of cultures of amoebae with single selected species of bacteria as well as cultures with a number of species of bacteria; the latter for production of cysts *in vitro*; cultures without bacteria but with *Trypanosoma cruzi*; with minced embryonic metazoan tissues and with cell-free homogenates of tissues; employment of specially devised techniques of biochemistry in studies on metabolism; use of techniques of microisolation and microculture; inoculation of germ-free mono-bacterial contaminated, autoclaved-killed bacterial contaminated, and "conventional" experimental animals with selected cultures defined above; and intravenous injection into check embryos and young mammals for production of hepatitis, and/or amoebic abscess of the liver or other organs.

Cooperation is extended to physicians in the Clinical Center of NIH in investigations of patients with amoebiasis by clinical methods as a basis for diagnosis, prophylaxis, and therapy.

9. (continued)

Major Findings:

Investigations on intravenous injection of Entamoeba histolytica into chick embryos near the time of hatching, and on intraportal injection of various mammals show that the amoeba may survive in the embryonic liver up to 24 hours and in the liver of mammals up to 48 hours without production of abscesses. For the first time, microcultures containing cell-free homogenates of chick embryo, inoculated with one washed amoeba per culture have allowed reproduction of amoebae(calculated average 11.4 generations/100 hrs). Also, for the first time, a method of accurately ascertaining the growth rate was developed. The rate of reproduction was found dependent on intracellular particles identical with or resembling mitochondria and not on association and/or symbiosis with other microorganisms. The factors appear to be heat-labile since they were not demonstrable in material heated up to 30 minutes at 50°C. However, supplementation of the heat-treated medium with a formula of thermostable vitamins, amino acids, and coenzymes has restored about two-thirds of the activity of heated medium. Medium free of particles could not be restored by the formula, suggesting that the amoeba is a phagotroph, that heating may have caused diffusion of growth factors into the liquid phase of the medium, and that factors from the added formula were adsorbed onto the heat-treated particles. It is probable however, that the particles contain one or more indiffusible thermo-stable growth factors. Certain characteristics of E. histolytica appeared to be affected by the selected microbial associates from which isolation is made from cell-free medium. Only half as many generations occurred with amoebae taken from T. cruzi cultures as compared with stock chick embryo mince-medium.

Collaborative experiments were continued at the Laboratory of Bacteriology of Notre Dame University where results previously announced indicated that amoebae from an inoculum of E. histolytica-T. cruzi did not survive in the germ-free gut of the guinea pig beyond 5 days, and did not produce lesions, although T. cruzi survived and entered the blood stream. Prior infection of the germ-free animal with single species of bacteria rendered the cecum suitable for survival of the amoeba, with resulting ulceration. Later experiments show that association or symbiosis with live bacteria is not required for survival and pathogenicity of the amoeba since survival of amoebae and ulceration of the cecum has occurred following feeding the germ-free guinea pigs with autoclaved feces of "conventional" guinea pigs, the results being attributed to heat-killed bacteria or products derived therefrom. Supplementation of the diet with autoclaved feces has also prevented the onset of what appears to be a viral infection that otherwise killed the animals.

Significance to Microbiological Research:

While it is generally accepted that E. histolytica is the causative agent of amoebic dysentery and/or extraintestinal amoebiasis, particularly abscess of the liver, little is known about the factors of infectivity and/or pathogenicity. The occurrence of so many cases of infection with

9. (continued)

the amoeba without demonstrable pathogenicity requires explanation. The occurrence of a rich flora of bacteria and fungi in man complicates experimentation on this host. The presence of bacteria in cultures generally used for inoculum is a complication that has been obviated with the use of bacteria-free cultures of the amoeba in germ-free animals. This method of investigation now allows for further study of the pathogenesis of the amoeba without the complication of associated organisms; for example, differences in strains of the parasite.

Proposed Course of Project:

The studies on metabolism of E. histolytica will be continued toward the goal of strictly axenic cultivation, in microcultures, and particularly in macrocultures where such pure cultures of the amoeba may be carried out, without resort to microisolation, and used for further information on pathogenicity, experimental chemotherapy, and immunodiagnosis. Analysis of the complex media thus far employed will be attempted so that inert ingredients may be eliminated with resulting definition of specific growth factors for the amoeba. Studies on changes induced by selected microbial associates will also be continued. Also problems of adaptation by this and other methods. The fruitful experiments on germfree guinea pigs will also be extended along lines indicated above. Continued collaboration with physicians at the Clinical Center is also on the agenda for more information on pathogenicity, chemotherapy, and immunodiagnosis in man - the natural host of E. histolytica.

Project Subtitle:

Genital Trichomoniasis.

Objectives:

The project is designed to investigate: (1) disturbances attributable to Trichomonas vaginalis in the urogenital tract of girls before puberty, sexually functional women before and during pregnancy and post partum, of women during and after menopause, and of those rendered sterile through surgery; (2) effects of other microorganisms such as Monilia albicans and/or species of bacteria that may act in symbiosis with the flagellate; (3) to what extent the trichomonad may be transmitted, by sexual intercourse or by fomites contaminated from the genitals, from T. tenax of the mouth or T. hominis of the intestinal tract which may not differ specifically from T. vaginalis; (4) incidences and epidemiology in women and men, particularly husbands and wives in patients at the Clinical Center and other hospitals within the Greater Washington area; (5) the possible relationship to malignancies; (6) the conditions in the genital tract of individuals not infected with the parasites for evaluation of the above-mentioned disturbances; (7) pharmaceutical preparations purported to cure trichomoniasis and/or moniliasis.

9. (continued)

Methods Employed:

Disturbances in the urogenital tract are investigated in clinical examinations including symptoms, inflammatory reactions and changes in vaginal physiology as indicated by pH, percentages of cornified and non-cornified cells, changes in glycogen content, secretions of mucus, and invasion of the vaginal lumen by leucocytes, as ascertained from vaginal specimens by direct microscopic examination, cultivation *in vitro*, and on stained slides. Particular reference is indicated to phases of the menstrual cycle and conditions occurring before puberty, after the menopause, or following surgery. Studies on transmission include comparative incidences in husband and wife, other household contacts, experiments with contaminated fomites and examination of specimens from the mouth and the intestinal tract. Other epidemiological investigations are conducted by means of questionnaires, interviews and examination of specimens as described above.

Data are obtainable from a large number of women employees of NIH, and other agencies of the Department of Health, Education and Welfare, through collaboration with the National Cancer Institute; from women patients and some men at NIH Clinical Center, Columbia Hospital for Women, and from physicians in private practice.

Major Findings:

Clinical investigations lend support to some published assertions on the pathogenicity of *T. vaginalis*. A number of symptoms and inflammatory reactions have been found in our surveys. However, many more data are needed to ascertain whether the disturbances are attributable to *T. vaginalis* alone. Methods of procedure have been instituted to ascertain the possible role of *T. vaginalis* in the incitation of malignancies.

Infection with *T. vaginalis* is 7 times more prevalent in Negro women employees at NIH than in white women, 64 and 9 percent respectively. At Columbia Hospital for Women a single examination of 16 husbands of infected wives, showed 6 cases positive. At NIH all infected women had viable *T. vaginalis* on the labia and a considerable number in the urethra. The flagellate was recovered on wash cloths used to cleanse the outer genitalia. More women had moniliasis than *T. vaginalis* and some had both infections. Infections of the mouth with *T. tenax* was less prevalent than of the urogenital tract with *T. vaginalis* but much higher than with *T. hominis* of the intestinal tract. The comparative prevalence of these organisms furnishes little support for the theory that *T. tenax* is identical with *T. vaginalis* and no support for the identity of *T. hominis* and *T. vaginalis*. Tentative results indicate that *T. vaginalis* may be transmitted to either sex through coitus and from woman to woman on fomites.

9. (continued)

Eight drugs thus far tested either singly or in combinations on a total of 16 women have failed to eliminate T. vaginalis. Some cases showed transient disappearance of the flagellate. The results with several drugs designed to cure moniliasis have been much more encouraging.

Significance to Microbiological Research:

Great importance of the project will be indicated if T. vaginalis is found to act as an incitant of malignancies of the urogenital tract.

Experiences of physicians with women patients having nervous disturbances because of genito-urinary disorders caused by or associated with infection with T. vaginalis have led to convictions that the flagellate is pathogenic. The gravity of the situation from the physician's point of view is evidenced by reports on the use more or less ineffectively of more than 200 chemotherapeutic agents. The current studies indicating the probability that the husband becomes infected, even though the original transmission may be other than coital, may be of fundamental importance in chemotherapy.

Proposed Course of Project:

Full advantage will be taken of the excellent opportunity for collaboration afforded by the project on Vaginal Cytology by the National Cancer Institute to study infections in employees at NIH and other agencies of the Department of Health, Education, and Welfare. Collaboration with physicians at the Clinical Center, other hospitals, and with private practitioners will also be continued. Continuation of other work discussed under headings as above is also contemplated. Since our biochemist has found full time employment in problems of amoebiasis, no investigations on metabolism of T. vaginalis are contemplated unless or until changes in the program appear desirable. However, the fundamental importance of such investigations is fully appreciated.

Project Subtitle:

Studies on Toxoplasmosis.

Objectives:

The aims of these studies are the solution of the problems of transmission of Toxoplasma, the elucidation of the biology of the parasite and of related organisms, and of strain differences, factors in susceptibility and immunity to toxoplasmosis, the manifestations and diagnosis of human toxoplasmosis, especially the ocular and lymphadenopathic forms, and the therapy and management of ocular toxoplasmosis as indicated by laboratory studies; also, the development of a diagnostic test practicable for routine laboratory use.

9. (continued)

Methods Employed:

The techniques are virological methods adapted for this fragile intracellular parasite. They include the range of animal inoculation procedures and in addition injections into the anterior and posterior chambers of the eye. Tissue cultures techniques are employed, and the unique in vitro dye test is used for titration of serum antibodies.

Collaborative clinical and laboratory studies are in progress with the Wilmer Institute of Johns Hopkins University and the Clinical Center of NIH. A similar project of lesser magnitude is conducted with the Eye Clinic of Walter Reed Army Hospital.

Major Findings:

Treatment of experimental toxoplasmic uveitis of rabbits by pyrimethamine and sulfadiazine indicates that the combination of these drugs is more effective in preventing the development of severe uveitis than either drug singly. The rabbit tolerates much larger doses of pyrimethamine than other animals, probably because of rapid detoxication. For the same reason, large doses of the drug are needed to accomplish the therapeutic effect.

An analysis has been accomplished of 200 unselected cases of endogenous uveitis studied in the collaborative project with the Wilmer Institute. The percentage of cases of granulomatous uveitis positive for Toxoplasma infection on the basis of the dye test was 61%, as compared to 19% for cases of non-granulomatous ocular disease. This adds strikingly to earlier statistical evidence supporting the thesis that Toxoplasma is an important etiology of blinding eye disease.

Tissue culture tests of drug action in vitro have shown that 1 gamma/ml of pyrimethamine inhibits proliferation of Toxoplasma and 3 gamm/ml destroys the parasite. Minimal effective concentrations of sulfadiazine were not accurately defined but were over 10X higher. The drugs combined were effective at concentrations of 0.1 gamma pyrimethamine and 5 gamma sulfadiazine per ml. Pyrimethamine is effective only against metabolizing parasites; suspensions of non-proliferating toxoplasmas were not discernibly affected by this drug at 4 gamma/ml.

A case of the lymphadenopathic form of human toxoplasmosis was studied very early in its course. Parasites were isolated from blood and lymph node, the time of appearance of antibodies was accurately noted, and response to pyrimethamine and sulfadiazine was good.

Antigens prepared from toxoplasmas killed by low heat, phenol, or formalin were found effective in producing an antibody response in guinea pigs, and in protecting the animals against challenge inoculations. However, the immunity is only a relative one, since challenging parasites given in small numbers are able to establish a systemic infection.

9. (continued)

Reptiles were found susceptible to infection with Toxoplasma. The parasites multiplied in terrapins and painted turtles inoculated intraperitoneally. This may have importance in relation to transmission in nature.

Infections are more easily established by feeding tissues from chronically infected animals than from animals in the acute stage. This is interpreted as indicating greater resistance of the pseudocyst than the proliferative form. It probably has bearing on the acquisiting of the infection by carnivores. However, the pseudocyst does not resist low heat (45°C) or drying.

Cross serological reactions against Toxoplasma have not been found in animals infected with *Eimeria tenella*, *Trypanosoma cruzi* or *Hepatozoon* sp.

Significance to Microbiological Research:

The statistical analysis of cases of granulomatous uveitis, especially chorioretinitis, in relation to immunologic evidence of Toxoplasma infection adds considerable support to the thesis that this parasite is a relatively frequent cause of ocular disease. We still need to find a method for the definitive diagnosis of ocular toxoplasmosis. In the absence of such a method it is difficult to evaluate therapy in human cases, although the results of therapeutic trials have been encouraging. The most important problem is the transmission of toxoplasmosis. These problems are in the realm of microbiological research.

From a basic standpoint, Toxoplasma offers opportunities for study of the cellular and humoral mechanisms of immunity, of genetic factors involved in the susceptibility of hosts and the resistance of strains, and of host-parasite relationships at the level of cellular metabolism.

Proposed Course of Project:

Attention will be devoted especially to the following points during the next calendar year: (1) The development of a practicable serological test for toxoplasmosis; (2) the development of techniques such as the use of tagged immune globulins for definitive diagnosis of human ocular toxoplasmosis; (3) the testing of drugs other than pyrimethamine and sulfadiazine in the control of experimental toxoplasmic uveitis of the rabbit; (4) the preparation of pure antigens for skin-testing and possibly for use in therapy of ocular infections, from tissue cultures of human cells; (5) the gathering of data, both experimental and observational, on possible modes of transmission; this involves considerable study of the specificity of serological tests which are the main criteria now for prevalence data; (6) the immunity produced by live agents and dead antigens; (7) the study of related parasites such as Besnoitia and Sarcocystis which possibly cause cross reactions influencing the specificity of diagnostic tests, and which may give leads as to the transmission of Toxoplasma and its pathogenesis.

Budget Data Sheet

10.

11.

12. BUDGET ACTIVITY:

RESEARCH

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE OR OTHER ORGANIZATION PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957.

Project Subtitle:

Studies on Amebiasis. The University of Notre Dame provides facilities for the germ-free animal studies.

Project Subtitle:

Genital Trichomoniasis. The Laboratory of Clinical Investigations personnel cooperate in the clinical studies.

Project Subtitle:

Studies on Toxoplasmosis. The National Institute of Neurological Diseases and Blindness provide the services of one GS-9 investigator.

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PHS (WITHOUT INTERCHANGE OF PERSONNEL FACILITIES OR FUNDS) IDENTIFY SUCH RESEARCH.

None

R. P. C. - 3
December 1955

Analysis of NIH Program Activities

Serial No. NMI - 59

Honors, Awards, and Publication Sheet

15.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

Ryan, R. W.; Hart, W. M.; Culligan, J. J.; Gunkel, R. D.; Jacobs, L.; and Cook, M. K.: Diagnosis and treatment of toxoplasmic uveitis. Trans. Am. Acad. Ophth. & Otolaryngol. 58: 867-884, Dec., 1954 (actually did not appear until Jan. 1955).

Jacobs, L.: Discussion of "Ocular lesions in hamsters" by J. K. Frenkel. Am. J. Ophth. 39: 225, Feb., 1955.

Rees, C. W.: Factors related to the pathogenicity of *Endamoeba histolytica*. Am. J. Gastroent. 23: 308-310, April, 1955.

Baernstein, H. D.: Aldolase in *Trichomonas vaginalis*. Exp. Parasitol. 4: 323-334.

Phillips, B. P.; Wolfe, P. A.; Rees, C. W.; Gordon, H. A.; Wright, W. H.; and Reyniers, J. A.: Studies on the ameba-bacterial relationship in amebiasis. Comparative results of the intracecal inoculation of germ-free, monocontaminated, and conventional guinea pigs with *Entamoeba histolytica*. Am. J. Trop. Med. & Hyg. 4: 675-692, July, 1955.

Jacobs, L.; Melton M. L.; and Cook, M. K.: Observations on toxoplasmosis in dogs. J. Parasit. 41: 353-361, August, 1955.

Rees, C. W.: Problems in Amoebiasis. 119 pp. Chas. C. Thomas, Springfield, Ill., September, 1955.

In Press

Jacobs, L.: Discussion of paper "The etiology of retinochoroiditis and uveitis," by Cassady, Culbertson, and Bahler. Am. J. Ophth.

Jacobs, L.: Newer knowledge of human toxoplasmosis. Am. J. Clin. Path.

Jacobs, L.; Ryan, R. W.; Hart, W. M.; and Cook, M. K.: The production of experimental ocular toxoplasmosis in rabbits. Proc. XVIIth Internat. Cong. Ophth.

Jacobs, L.: Propagation, morphology, and biology of *Toxoplasma*. Ann. N. Y. Acad. Sci.

Hoover, R.; Naquin, H.; Jacobs, L.; Woods, A. C.; Gans, J.; and Wood, R. M.: A further study of the role of toxoplasmosis in the etiology of endogenous uveitis. Trans. Am. Acad. Ophth. & Otalryngol.

16. (continued)

Brown, J. and Jacobs, L.: Adult toxoplasmosis: Report of a case due to laboratory infection. Ann. Int. Med.

17. HONORS OR AWARDS FOR THE PROJECT

Leon Jacobs received Alumnus Award of Honor from Brooklyn College, November 1955.

Leon Jacobs was named Representative of American Society of Tropical Medicine & Hygiene to National Research Council for 1956-1959.

Leon Jacobs was elected Editor, Journal of Parasitology.

Bruce F. Phillips received the Superior Accomplishment Award, DHEW, 1955.

R. P. C. - 1 Analysis of NIH Program Activities
December 1955

Project Description Sheet

1. National Microbiological Institute
INSTITUTE

2. Laboratory of Tropical Diseases
LABORATORY OR BRANCH

3. Medical Entomology
SECTION

4. Bethesda
LOCATION

5. NMI - 60
SERIAL NO.

6. Studies of Arthropods, Their Control, and Virus-arthropod Relations.
PROJECT TITLE

7. Dr. Paul A. Woke
PRINCIPAL INVESTIGATOR

8. Dr. W. H. W. Komp, Mr. H. T. Dalmat, and Dr. J. C. Jones
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Project Subtitle:

Taxonomy and Biology of Neo-tropical Culicidae.

Objectives:

To describe new species, set in order previous descriptions and classifications, and record biological information on neo-tropical Culicidae.

Methods Employed:

Morphological studies reveal structures differentiating species and subspecies; literature studies together with newer knowledge of structures and relationships reveal ways in which relationships of species can be more accurately defined; observations during field studies reveal habits and behavior useful in differentiating species and in control.

Major Findings:

Information on taxonomic status, geographical distribution, breeding places, biting habits, and relation to yellow fever of several species of Haemagogus; and information on the taxonomy and biology of over 70 species of mosquitoes from an endemic area of yellow fever in Colombia was compiled and prepared for publication.

Significance to Microbiological Research:

Since yellow fever, malaria, filariasis, and other mosquito-borne diseases continue to be of world-wide importance, and encephalitides are important in the United States, increased knowledge of mosquitoes is of public health significance.

9. (continued)

Proposed Course of Project:

This project was terminated by the death of the investigator on December 7.

Project Subtitle:

Arthropod Transmission of Shope's Cottontail Fibroma.

Objectives:

To determine (1) the mechanism of transmission of Shope's virus fibroma of cottontails; (2) whether there is propagation of the virus in the arthropod vector or whether it is transmitted by purely mechanical means; (3) the actual vectors of the disease in nature; and to (4) develop a strain that will be transmittable by arthropods from domestic rabbit to domestic rabbit with a view of producing cross-immunity in rabbits to infection with myxomatosis.

Methods Employed:

The virus is isolated from tumors of cottontails, prepared in suspensions which are introduced into arthropods either orally or by inoculation, so that controlled studies of the pathogenic agent in the vectors can be made. The arthropods are also permitted to feed on tumors of various ages, after which they are induced to feed on normal hosts to which they transmit the infection. Titrations of tumor suspensions or of suspensions of arthropod tissue are made by serial intracutaneous inoculations into rabbits. The electron microscope is also being employed as a means of determining viral content in the arthropods.

Major Findings:

Freely feeding *Aedes aegypti* transmit to cottontails fibromas that are similar in appearance to those of natural occurrence. Experimentally induced fibromas can persist on cottontails for at least 10 months without the loss of virus titer, and on wild cottontails for at least 6 months, suggesting that these animals can serve as effective reservoirs of infection. Infected mosquitoes will maintain high titers of virus at least up to 5 weeks, in spite of repeated feedings on normal hosts. The virus is localized primarily amongst the stylets of the mouthparts, a good concentration in the clypeal region, but none usually being found in the head capsule, thorax, or abdomen. Transmission by mosquitoes has been accomplished from white rabbit to white rabbit, suckling animals giving results closer to those of the natural cottontail host. Neither carcinogens nor cortisone appeared to enhance tumor development or virus titer.

9. (continued)

Significance to Microbiological Research:

The magnitude of the cancer problem warrants the development of every possible approach which might throw light on the etiology and mechanism of growth of neoplasms. It is possible that studies of neoplasms of lower animals may offer an advantageous approach to some of the problems encountered in man. Shope's fibroma of rabbits has been found occurring naturally in several localities east of the Mississippi River and material is therefore available for study. It is possible that arthropods may play a role in the transmission of other neoplastic diseases.

Proposed Course of Project:

Continued use of present techniques and the introduction of a few additional ones to determine whether or not arthropod transmission is purely mechanical or whether there is virus multiplication in the vector species. Reduviid bugs and chiggers will also be tested for their ability to transmit. Recent epidemiological evidence indicates that chiggers may be more likely vectors of this virus in nature. When electron microscope techniques are refined for use in viewing the virus in the mosquito mouthparts, this tool will be used in determining the actual number of virus particles at various stages in experiments designed to show whether transmission is mechanical or biological. By developing less virulent strains of the virus, it is hoped that more constant success will be achieved in establishing transmission from domestic animal to domestic animal. It is also expected that serological testing of the wild rabbit population at the Patuxent Research Refuge will be inaugurated in an attempt to learn the prevalence of the disease.

Project Subtitle:

Pathogen-Arthropod Host Relation Studies with Special Reference to Survival and Multiplication of Viruses in Arthropod Tissues.

Objectives:

To develop media suitable for the culture of arthropod organs and tissues as a basis upon which to investigate the possibilities of survival and multiplication of viruses in such cultures as well as in living arthropods, and to determine possible transmission of certain viruses by arthropods.

Methods Employed:

Organs and tissues are incubated in culture media of numerous varied formulations in a search for media that are most suitable

9. (continued)

for maintaining survival and normal activity of tissues. Virus is injected into the hemocoele, incubated for varying periods of time at different temperatures, then the ground bodies of the arthropods are inoculated intracerebrally into test mice to determine survival. Transmission is determined mainly by allowing arthropods to bite healthy animals after feeding on virus or on sick animals, and by force-feeding feces of infected arthropods, and the arthropods themselves to mice.

Major Findings:

The virus of encephalomyelitis of mice was found to survive for three weeks at 30° C. and 90% RH in the conenose bugs, Rhodnius prolixus, Triatoma phyllosoma pallidipennis, and Triatoma infestans, and for at least three weeks at 35° C. and 90% RH in crickets, one week in the snail, Lymnaea stagnalis, and two weeks in the snail, Australorbis glabratus.

Three species of triatomes did not transmit mouse poliomyelitis by biting and bloodsucking or through excreta. Mice were shown to be susceptible to the virus inoculated into the bloodstream but not when injected subcutaneously.

Excised organs of the mosquito, Aedes aegypti, have survived in modified Ball's tissue culture medium by the roller tube method as follows: Hindgut, 27 days (contracted 10 days); ovary, 24 days (contracted 3 days); midgut, 27 days; foregut, 13 days; malpighian tubules, 24 days.

Extracts from the southern armyworm, Prodenia eridania, have not proven more suitable than chicken or horse serum in tissue culture media.

The use of 2, 3, 5-triphenyltetrazolium chloride has proven suitable as an objective method for determining the duration of survival of organs and non-proliferating tissues in tissue culture by distinguishing between living and dead tissues.

Iodine in potassium iodide and sodium hypochlorite, suitably controlled as to concentration, time of action, and time of application, proved acceptable as a fungicidal agent for use externally on mosquito pupae that are to be employed in tissue culture.

Significance to Microbiological Research:

The results already achieved aid in development of culture media for arthropod tissues. When suitable means are available for maintaining intact organs and tissues for prolonged periods in artificial media and for culturing arthropod tissues, studies can be made on the possible cultivation of viruses such as that of poliomyelitis in arthropod tissue culture, identification of

9. (continued)

the specific tissues that support virus multiplication, determination of potential vectors in tissue culture, and virus-arthropod tissue relations.

Proposed Course of Project:

Using the best available knowledge of the requirements of arthropod tissues and trial and error procedures, a new tissue culture medium will be developed starting with the saline base and progressing with thorough studies from group to group of the different tissue and hemolymph components until a presumably complete and balanced medium is attained.

Project Subtitle:

Studies on the Mode of Action of Insecticides with Special Reference to Development of Resistance.

Objectives:

To define the inherent resistance of Anopheles larvae to DDT and to attempt to raise or lower this level.

Methods Employed:

Fourth instar Anopheles quadrimaculatus of known age and sex are individually exposed to freshly prepared acetone suspensions of DDT 10^{-4} under controlled conditions of light, temperature, and humidity. Depending upon age they are exposed for 1, 2½, 5, 10, 15, or 30 minutes to DDT then washed in two changes of distilled water (1,000 ml. per change) and placed in individual beakers for subsequent observations on their development.

Major Findings:

(1) Techniques for roughly defining levels of resistance to poisons within less than a day have been devised.

(2) Highly accurate means for differentiating sexes in larvae and for precise determinations of chronological age have been developed.

(3) To date, the levels of inherent DDT resistance of fourth stage Anopheles quadrimaculatus Say larvae show 10 to 100 fold variations in mortality when strain, sex, age, temperature, humidity, light, concentration of DDT, and exposure time are rigidly controlled. So far, there is no explanation for this wide variation.

9. (continued)

Significance to Microbiological Research:

If the present data prove to be correct, this would mean that unless extremely great increases occur in DDT resistance, they could not be detected since 100 fold variations may occur by chance under rigidly controlled conditions. Nevertheless, under field (natural) conditions, it is possible to accurately and rapidly assess the resistance in a given sample of larvae so that adequate calculations as to dose applications could be made at a given time for a given area.

Variations due to both age and sex of mosquito larvae, if uncontrolled, are sufficient to lead to serious misinterpretations of data. Current work shows the importance of their control especially with regard to bioassay techniques which are widely used throughout the world.

Proposed Course of Project:

To continue current experiments and statistical assessment of levels of DDT resistance in *Anopheles* larvae and pupae.

Since it is suspected that the large variation found in inherent resistance to DDT in the LTD strain of *Anopheles quadrimaculatus* Say results from equally wide variations in the genetical patterns between different larval groups, plans for the future include a possible significant reduction in genetical variations by inbreeding highly resistant larvae and testing their offspring.

Budget Data Sheet

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12. BUDGET ACTIVITY

RESEARCH

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATION PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 OR 1957.

Project Subtitle: Arthropod Transmission of Shope's Cottontail Fibroma: The Wildlife Pathology Section of the Patuxent Research Refuge supplies naturally-infected cottontails; the Missouri State Conservation Department supplies wild cottontail rabbits; the Laboratory of Physiology, NCI, does the electron microscope studies; and the Clinical Pathology Section, NCI carries out pathological studies when these are considered necessary.

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PHS (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS) IDENTIFY SUCH RESEARCH.

None

Honors, Awards, and Publication Sheet

15.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

Jones, J. C.: Thoracic vesicles in Anopheles larvae. Mosq. News 15 (3): 168-170.

Kilham, L. and Dalmat, H. T.: Host-virus-mosquito relations of Shope fibromas in cottontail rabbits. Am. J. Hyg. 61 (1): 45-54. January, 1955.

Komp, W. H. W.: Notes on Haemagogus iridicolor Dyar. (Diptera, Culicidae). Proc. Ent. Soc. Wash., 57: 29-31. February 1955.

Komp, W. H. W.: Notes on the larva of Haemagogus janthinomys Dyar (Diptera, Culicidae). Proc. Ent. Soc. Wash., 57: 137. June 1955.

Komp, W. H. W.: The occurrence of Haemagogus boshelli in Panama (Diptera, Culicidae) Proc. Ent. Soc. Wash., 57: 181-182. August 1955.

Komp, W. H. W.: The oviposition of Haemagogus equinus in nature (Diptera, Culicidae) Mosquito News 15 (3): 163-164. September 1955.

Komp, W. H. W.: The breeding of Haemagogus argyromeris D. and L. in saline water (Diptera, Culicidae). Mosquito News 15 (3): 179-180. September 1955.

Komp, W. H. W.: Notes on the larva of Haemagogus panarchys Dyar (Diptera, Culicidae). Proc. Ent. Soc. Wash., 57 (5): 237-239. October 1955.

Komp, W. H. W.: The taxonomic status of Haemagogus janthinomys Dyar (Diptera, Culicidae). Proc. Ent. Soc. Wash., 27 (6): 277-280, December 1955.

Woke, P. A.: Deferred oviposition in Aedes aegypti (Linnaeus) (Diptera, Culicidae). Ann. Ent. Soc. Amer., 48 (1-2): 39-46. January-March 1955.

Woke, P. A.: Aspirator-cage combinations for delicate and infected arthropods. Ann. Ent. Soc. Amer., 48 (6): November 1955.

16. (continued)

In Press

Jones, J. C.: A study of normal heart rates in intact Anopheles quadrimaculatus Say larvae. J. Exp. Zool.

Jones, J. C. Notes on a unispiraculate Anopheles quadrimaculatus Say larva. Proc. Ent. Soc. Wash.

Komp, W. H. W.: Notes on mosquitoes from an area of endemic yellow fever in Colombia. Proc. Ent. Soc. Wash.

Komp, W. H. W.: Copulation in crab-hole mosquitoes. Proc. Ent. Soc. Wash.

Woke, P. A.; Ally, M. S., and Rosenberger, C. R. Jr: The numbers of eggs developed related to the quantities of human blood ingested in Aedes aegypti (Linnaeus) (Diptera, Culicidae). Ann. Ent. Soc. Amer.

17. Honors

Dr. Jack C. Jones was elected a member of the American Society of Zoologists.

W. H. W. Komp received the honorary degree of Doctor of Science from Rutgers University on October 6, 1955.

Dr. W. H. W. Komp was re-elected to the Scientific Advisory Board of the Gorgas Memorial Institute, Oct.

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December 1955

Project Description Sheet

1. National Microbiological Institute 2. Laboratory of Tropical Diseases
INSTITUTE LABORATORY OR BRANCH

3. Physiology 4. Bethesda 5. NMI- 61
SECTION LOCATION SERIAL NO.

6. Fundamental Physiological and Biochemical Studies on Parasites, Intermediate Hosts, and Parasitized Animals.
PROJECT TITLE

7. Dr. Theodor von Brand
PRINCIPAL INVESTIGATOR

8. Dr. E. C. Weinbach, Dr. W. L. Newton, Dr. T. I. Mercado, Mrs. E. J. Tobie
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Project Subtitle:

Physiology and Biochemistry of Parasites.

Objectives:

The objective of this part of the program is to gain insight into the metabolic activities of parasites, especially members of the family Trypanosomidae.

Methods Employed:

Whole organisms, homogenates, or isolated cell fractions are incubated in appropriate media and assayed, depending upon the aim of a particular experiment, for respiratory activity, consumption of a given substrate, accumulation of metabolic end products or presence and activity of a certain enzyme. Polysaccharides isolated from pathogenic organisms are studied by paper electrophoresis and products of hydrolysis identified by paper chromatography.

Major Findings:

The demonstration, characterization, and intracellular distribution of succinic dehydrogenase in *Trypanosoma cruzi*. The demonstration, 50-fold purification and characterization of TPN-linked isocitric dehydrogenase from *T. cruzi*. The enzyme differs from the mammalian one in its greater affinity for Mn^{++}; purified preparations of the protozoan enzyme having an absolute requirement for this cation. The polysaccharide of *T. cruzi* was found to be electrophorectically homogeneous and on acid hydrolysis yielded galactose, mannose, and xylose,

9. (continued)

Significance to Microbiological Research:

In order to obtain an understanding of the pathogenesis of human parasites, it is essential to obtain additional knowledge concerning their metabolism, because it is an integral part of the host-parasite complex. Furthermore, a detailed knowledge of the metabolic patterns and pathways of parasites will lay a foundation for a rational approach to chemotherapy in contrast to the prevailing empirical one.

Proposed Course of Project:

A study of alternative pathways of carbohydrate metabolism (e.g. hexose monophosphate shunt) of parasites with special emphasis on pyruvate metabolism will be undertaken. Study of the chemical composition of parasites will be extended to include qualitative and quantitative estimation of lipid content.

Project Subtitle:

Physiology and Biochemistry of Intermediate Hosts.

Objectives:

The objective of this part of the program is to elucidate the basic metabolic relations of snails transmitting blood flukes, to study the mechanism of action of molluscacides, and to investigate whether snails will develop resistance to molluscacides.

Methods Employed:

In cooperation with the Section on Helminthic Diseases, desiccation studies of snails are conducted at various relative humidities. Heart rate and respiration are followed during this desiccation. Polysaccharides isolated from various snail species are examined for homogeniety by paper electrophoresis and their hydrolysis products separated and identified by paper chromatography. Enzyme systems are isolated (disruption of cells, separation of subcellular particulates), and the influence of pentachlorophenol and related phenols on cellular reactions synthesizing and transferring high energy compounds is studied. Successive generations of snails derived from selected survivors of gradually increased concentrations of pentachlorophenol are tested for changes in susceptibility.

Major Findings:

During desiccation at relatively high humidities, respiration rate rapidly decreased to a constant level with a concomitant decrease in heart rate. At low humidities, the steady respiratory

9. (continued)

decrease was accompanied by a weak heartbeat at an accelerated rate. Snail polysaccharide was separated electrophoretically into two components. On hydrolysis, one component yielded only glucose; other than glucose, the second component yielded a mixture of monosaccharides whose exact composition apparently is specific for the species and tissues employed. The demonstration of oxidative phosphorylation for the first time in snail tissue and the complete uncoupling of this endergonic process by low concentrations of pentachlorophenol. Australorbis continues to show a slight, but definite, increase in resistance to pentachlorophenol.

Significance to Microbiological Research:

The studies are in part a contribution to comparative biochemistry, and in part have direct implications on practical problems. The elucidation of the mode of action of molluscacides should help to pave a way towards the rational development of molluscacides. The question whether snails can develop resistance to molluscacides is of evident importance in view of the difficult situation encountered by entomologists with insecticide resistance in flies and other insects.

Proposed Course of Project:

The study of the chemical composition of intermediate hosts will be terminated early in 1956. Personnel assigned to this project will undertake a study of the chemical composition of parasites and will intensify their investigations of the physiology and biochemistry of desiccation (in cooperation with the Section on Helminthic Diseases). It is planned to continue the study of the mode of action of pentachlorophenol with emphasis on its interference with phosphate metabolism. The study of resistance will continue.

Project Subtitle:

Pathological Physiology.

Objectives:

The objective of this part of the program is to elucidate the pathogenic action of parasites on their vertebrate hosts, and the behavior of parasites in vivo.

Methods Employed:

Infected (Plasmodium berghei) and non-infected rats fed on fructose are treated with cortisone and related steroids, and the glycogen synthesized in vivo is assayed quantitatively and histochemically. Mice and rabbits, infected with pathogenic trypanosomes,

9. (continued)

are treated with subcurative doses of antibiotics. Following each of a series of relapses, they are again so treated and histopathological studies done in cooperation with the Laboratory of Pathology and Histochemistry. Dogs infected with Dirofilaria immitis are observed for microfilarial periodicity and subjected to various experimental procedures altering the physiological state in order to determine the mechanism of such periodicity.

Major Findings:

Quantitative and histochemical studies showed that the injection of cortisone acetate causes a significant increase in the synthesis of liver glycogen in rats infected with Plasmodium berghei. The antibiotic T3018 was found very effective against T. congolense in contrast to Puromycin and its amino nucleoside. Pathological changes in the central nervous system of the mouse, comparable to those produced by trypanosomiasis in man, were produced by prolonging the course of the experimental infection by administration of subcurative doses of the amino nucleoside of Puromycin.

Adrenaline and histamine failed to affect the concentration of microfilariae of Dirofilaria immitis in the peripheral circulation, whereas insulin increased the concentration in one dog and not in another. An incidental finding was that an as yet unidentified microfilaria was observed in the peripheral circulation of 7% of dogs from an area in Penssylvania.

Significance to Microbiological Research:

The studies are designed to clarify the pathogenesis of parasites. Moreover, such a knowledge is of considerable significance in the development of new therapeutic procedures.

Proposed Course of the Project:

The study of the influence of cortisone on glycogen synthesis in normal and infected (P. berghei) rats will be terminated early in 1956. It is planned to then initiate comparative histochemical and biochemical studies of liver enzymes involved in carbohydrate metabolism of uninfected rats and those infected with P. berghei. The studies involving the cultivation of refractory trypanosomes will be expanded to include the development of special media suitable for our particular physiological studies. It is proposed to study the resistance of experimental animals to reinfection with trypanosomes following their cure with antibiotics. The study of microfilarial periodicity will be continued in an effort to elucidate the physiological basis of the phenomenon.

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December 1955 Analysis of NIH Program Activities Serial No. NMI - 61

Budget Data Sheet

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12. BUDGET ACTIVITY:

RESEARCH

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATION PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957.

None

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PHS (WITHOUT INTERCHANGE OR PERSONNEL, FACILITIES OR FUNDS) IDENTIFY SUCH RESEARCH.

None

Honors, Awards, and Publications Sheet

15.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

von Brand, T., McMahon, P., and Nolan, M. O.: Observations on the postanaerobic metabolism of some fresh-water snails. Physiol. Zool. 28: 35-40, January 1955.

Newton, W. L., and von Brand, T.; Comparative physiological studies on two geographical strains of Australorbis glabratus. Exper. Parasitol. 4: 244-255, May 1955.

von Brand, T., Weinbach, E. C., and Tobie, E. J.: Comparative studies on the metabolism of the culture form and the bloodstream form of Trypanosoma gambiense. J. Cell. and Comp. Physiol. 45, 421-433, June, 1955.

Newton, W. L.: The establishment of a strain of Australorbis glabratus which combines albinism and high susceptibility to infection with Schistosoma mansoni. J. Parasitol. 41, 526-528, October, 1955.

Agosin, M., and von Brand, T.: Characterization and Intracellular Distribution of the Succinic Dehydrogenase of Trypanosoma cruzi. Exptl. Parasit. 4, 548-563, November 1955.

von Brand, T.: Anaerobiosis in Australorbis glabratus: Temperature effects and tissue hydration. J. Wash. Acad. Sciences, 45, 373-377, December 1955.

In Press:

Agosin, M. and Weinbach, E. C.: Partial purification and characterization of the isocitric dehydrogenase from Trypanosoma cruzi. Biochem. et Bioph. Acta.

von Brand, T. and Agosin, M.: The utilization of Krebs cycle intermediates by the culture forms of Trypanosoma cruzi and Leishmania tropica. J. Infect. Diseases.

von Brand, T. and Mercado, T. I.: Quantitative and histochemical studies on glycogenesis in the liver of rats infected with Plasmodium berghei. Exptl. Parasitol.

16. (continued)

Mercado, T. I.; Trembley, H. L., and von Brand, T.: Observations on the oxygen consumption of some adult mosquitoes. Physiol. Comp. et Oecol.

Tobie, E. J. and Highman, B.: Influence of the amino nucleoside of puromycin on the course and pathology of trypanosome infections in rabbits and mice. Am. J. Trop. Med. & Hyg.

Weinbach, E. C. and Nolan, M. O.: The effect of pentachlorophenol on snail metabolism. Exptl. Parasitol.

Weinbach, E. C.: Pentachlorophenol and mitochondrial adenosinetriphosphatase. J. Biol. Chem.

17. Honors:

Dr. Theodor von Brand was made Honorary Member of the Medical Faculty, University of Chile, on December 6, 1955. Acceptance of this honor conferred upon Dr. von Brand the status of an Honorary Full Professor in the Faculty.

R. P. C. - 1 Analysis of NIH Program Activities
December 1955

Project Description Sheet

1. National Microbiological Institute
INSTITUTE

2. Laboratory of Tropical Diseases
LABORATORY OR BRANCH

3. Immunology
SECTION

4. Bethesda
LOCATION

5. NMI -62
SERIAL NO.

6. Studies on the Mechanism of Allergic and Immunologic Phenomena in Parasitic Diseases.
PROJECT TITLE

7. Mr. John Bozicevich
PRINCIPAL INVESTIGATOR

8. Dr. J. E. Tobie and Miss E. H. Thomas
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Project Subtitle:

The Nature of the Immune Response in Parasitic Diseases

Objectives

To determine the mechanisms involved in antibody formation by histological and cytological methods and to separate the active immunological components by fractionation of the antigen; and to determine the specific localization of the antigen within the tissues of the host.

Methods Employed:

Two methods will be employed for the determination of the localization of the antigen and antibody production. (A) Antibodies will be "tagged" with fluorescein and the tissues of immunized or infected animals will be bathed with the tagged component to determine the specific uptake of the fluorescein. The reverse of this procedure will also be utilized. In this instance the antigen will be tagged and injected into sensitized and infected animals to determine the localization and specific uptake of the antigen. (B) Antibodies will be tagged with radioactive iodine and injected into animals to determine the specific uptake of the labeled antibody. *In vitro* tests will be conducted utilizing the radioactively tagged anti-serum and antigen for quantitative precipitin studies with soluble antigen and with viable organisms as antigen.

9. (continued)

Major Findings:

Project A has been recently initiated. No findings can be reported at the present time. However, the antisera and the fluor have been prepared in order to carry on further studies.

Project B. Radioactive labeled anti-rat kidney gamma globulin injected into rats has shown a specific uptake of the tagged antiglobulin in the kidney. This is contrary to the findings of Bale and Spar who reported that the adrenals and ovaries took up more of the labeled antibody than the kidney. Further work will be done in order to clarify this discrepancy.

Quantitative precipitin tests with labeled anti-*Trypanosoma cruzi* gamma globulin with soluble antigen have shown that in the zone of equivalence, as determined by complement fixation tests, the nitrogen in the precipitate of immune tagged gamma globulin is only slightly greater than that in normal tagged gamma globulin. In other words the increase in the nitrogen content of the specific precipitate from the immune gamma globulin is not of the order to be significant. The radioactive counts likewise were not significant when the soluble antigen was employed. When viable organisms were used as the antigen it was again found that the quantitative nitrogen determinations between the anti-gamma globulin and the normal gamma globulin were not significantly different. However, the radioactive counts obtained when the organisms were placed in the labeled anti-*T. cruzi* gamma globulin were approximately twice that obtained from the normal labeled gamma globulin. This would indicate that the organisms had removed from the anti-gamma globulin a fraction which is not nitrogenous but, perhaps, lipoidal in character.

Significance to Microbiological Research:

The cytological localization of the soluble antigen and of the organisms might provide a clue which would be of value in the selection of drugs for chemotherapeusis. It is hoped that information might be obtained on the function of the reticuloendothelial system in immunity and in hypersensitivity.

Hypersensitivity in parasitic diseases has received slight attention in the past even though marked allergic states are developed to certain parasites. By employing the sensitizing factors in antigens obtained from parasites it may be possible to understand the responses shown by allergic individuals.

9. (Continued)

Proposed Course of Project:

During the coming year a great deal of time will be devoted to labeling antibodies and antigens for study with the aid of fluorescence microscopy. Comparisons of the localization of the antigen *in vitro* and *in vivo* will be continued. It is anticipated that studies will also be performed on germ-free animals and animals raised under natural conditions. Fluorescent tagging will also be employed in the study of allergy.

Gamma globulin fractions prepared by chemical separation and by physical separation will be tagged with radioactive iodine and studied both *in vitro* and *in vivo*. Electrophoretic studies on these fractions will be conducted. Antigens will be fractionated by chemical and physical means and these fractions will also be labeled and tested both *in vivo* and *in vitro*. The labeled fractions of the antigen will be employed as intradermal antigens on hypersensitive and infected animals to determine the specific exciting factor in the antigens. Study of the blood proteins of normal and infected animals will be undertaken. The study of the humoral constituents of hypersensitive animals will likewise be studied.

Project Subtitle:

Studies on the Role of the "Accessory Factor" in the Performance of the Dye Test for Toxoplasmosis.

Objectives:

To isolate and determine the factor responsible in normal human serum which contains the accessory factor employed in the dye test. To recover this factor from animal sera and purify the material in order to substitute it for human serum for the performance of the dye test.

Methods Employed:

Fractionation of normal human serum by various means will be undertaken. Each fraction will be employed in the dye test to determine its efficacy. Preparation of active "zymosan" will be continued.

Significance to Microbiological Research:

If a stable "accessory factor" can be obtained the cost of the dye test for toxoplasmosis will be greatly reduced. Furthermore,

9. (continued)

our knowledge of the components employed as "accessory factor" may furnish us with basic information on the mechanism of natural immunity and hypersensitivity.

Extraction of the properdin from animals and normal serum may be of value in the study of the allergic mechanism in hypersensitive individuals.

Major Findings:

There are no valid findings at the present time. A great deal of difficulty has been encountered in preparing a satisfactory zymosan for the removal of the active fraction from the serum "properdin." Initially, studies of normal plasma to which zymosan had been added and the properdin eluted and employed as an accessory factor had shown some promise. It was discovered, however, that this extract was not as efficient as accessory factor obtained from normal usable serum.

Proposed Course of the Project:

Chemical fractionation and fractionation by absorption with zymosan will be continued. These fractions will be tested by the dye test with and without the presence of complement. Electrophoretic separation of serum will be attempted and the fractions will be tested for activity.

Project Subtitle:

Study of the Role of "Serotonin" and Histamine in Allergic Responses.

Objectives:

To determine the components of parasite extracts responsible for the increase in histamine and serotonin in immunized animals.

Methods Employed:

To immunize animals to various fractions of parasite extracts and to conduct in vitro tests for increase in serotonin and histamine. To also conduct in vivo tests on hypersensitive animals for the increase of serotonin and histamine by employing "micro-shock."

Major Findings:

This project has been recently initiated. However, preliminary findings using the heparinized whole blood of animals

9. (continued)

sensitized with egg albumin and with ascaris powder has failed to show an increase in serotonin by the *in vitro* method. Humphrey and Jaques reported an increase in serotonin from blood platelets when the antigen-antibody reaction was performed. These authors employed the micro-shock technic and it is possible that this reaction may be evident *in vivo* only.

Significance to Microbiological Research:

The findings of Humphrey and Jaques need to be confirmed. It may be possible to better understand the role of serotonin and histamine in allergic diseases.

Proposed Course of the Project:

Animals will be immunized to various antigens and fractions prepared by chemical and physical means and tested both *in vitro* and *in vivo* for the increase of serotonin and histamine. Fractions of the antigen will be labeled with radioactive iodine and the above studies paralleled with radioactive counts both *in vitro* and *in vivo*.

Budget Data Sheet

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12.

BUDGET ACTIVITY:

RESEARCH

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATION PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PHS (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS) IDENTIFY SUCH RESEARCH.

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December 1955
Honors, Awards, and Publications Sheet

15.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

(1) Bozicevich, John: Serological considerations relative to the diagnosis of amoebiasis. Am. J. Gastroent., 23, 332-334, April 1955.

(2) Tobie, John E.: Parasitological considerations relative to the diagnosis of amebiasis. Am. J. Gastroent., 23, 329-331, April 1955.

17. HONORS OR AWARDS FOR THE PROJECT

Mr. John Bozicevich was elected a fellow of the New York Academy of Sciences on December 1, 1955 in recognition of his contributions to parasitology.

R. P. C. - 1 Analysis of NIH Program Activities
December 1955

Project Description Sheet

1. National Microbiological Institute
INSTITUTE

2. Laboratory of Tropical Diseases
LABORATORY OR BRANCH

3. Chemotherapy
SECTION Unit

4. Bethesda
LOCATION

5. NMI - 63
SERIAL NO.

6. Chemotherapy of Parasitic Diseases
PROJECT TITLE

7. Dr. G. Robert Coatney
PRINCIPAL INVESTIGATOR

8. Dr. J. Greenberg, Dr. H. W. Bond, Dr. J. Sherman, Dr. G. Luttermoser, and Miss D. J. Taylor
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Project Subtitle:

Drug Resistance in Plasmodia

Objectives:

To study the methods by which resistance to therapeutic agents is acquired by animal parasites; the stability of resistance once acquired; its heritability; the interaction between susceptible and resistant parasites through the sexual cycle; the relationship between resistance and other measurable biological properties of the parasite such as sexuality and tissue preference. Conversely, to study the influence of heritable characteristics of the host on the biological properties of the parasite.

Methods Employed:

Using *Plasmodium gallinaceum*, strains are developed with unique combinations of properties involving drug-resistance, virulence, and sexuality. These strains are hybridized systematically and recombinations recovered and analyzed. *P. berghei* is used in an inbred strain of mice and their hybrids. Tissue preferences of the parasite in these animals is observed and compared.

9. (continued)

Major Findings:

A strain of Plasmodium gallinaceum made resistant to Daraprim was: (a) less virulent than the parent sensitive strain i.e., fewer chicks died of exoerythrocytic forms of the parasite, (b) slower growing than sensitive strains and (c) able, after a sufficient length of time (3 weeks), to produce gametocytes. Passed serially through mosquitoes this strain progressively lost virulence, at variable rates from line to line, until after 5 or 6 passages the strain was completely benign. Drug resistance is not necessary for this series of events because some sensitive strains will also lose virulence on serial mosquito passage. Passage through mosquitoes does not inevitably produce the same results since the SP strain has retained its ability to proliferate in the fixed tissues and kill chicks after over ten years of mosquito passage.

Inbred strains of mice differed in the degree to which mature erythrocytes were invaded by P. berghei, but there were no differences in the rate of invasion of immature erythrocytes. The ability to limit the invasion of mature erythrocytes was one of several major determinants of survival, and was influenced in a complicated way by the genetic constitution of the host and by environmental factors.

Passage of the parasite through certain strains of mice resulted consistently in marked loss of ability to invade mature erythrocytes. The change was not permanent when the parasite was transferred to other strains.

Significance to Microbiological Research:

From a practical point of view, information on drug resistance is of real value to those interested in programs of mass therapy. From the viewpoint of basic research the plasmodia are invaluable tools in studies involving the genetics of microorganisms. Very few other pathogenic organisms have a sexual cycle, so the genetic transfer of drug resistance can only be inferred and cannot be studied in terms of number of genes involved and the location and interrelationship of such genes. The inheritance of such factors as pathogenicity, sexuality, and tissue preferences cannot be studied in other protozoan or bacterial pathogens. As a result this whole field has been neglected. Many of the above characteristics are intimately related to drug activity. For example, the therapeutic failure of such compounds as quinine is now known to be related to its inability to kill parasites in certain stages of their development. There is reason to suppose that plasmodia are not unique in this respect. Furthermore, it has been shown that the virulence of plasmodia can be modified by certain experimental procedures. The practical and theoretical implications of these observations are readily apparent.

9. (continued)

Proposed Course of Project:

This project will be discontinued in favor of basic studies on the mode of action of therapeutic agents with particular emphasis on Puromycin, in line with the redirection of the program of the entire Section.

Project Subtitle:

Chemical Pharmacology and Toxicology.

Objectives:

To determine basic pharmacological and toxicological properties of therapeutic compounds as well as methods for the assay of such compounds and their degradation products in biological systems, their distribution, degradation and excretion, and preferred methods of administration.

Methods Employed:

Standard pharmacological methods are used to determine the toxicity and pharmacodynamics of compounds in various hosts and their effect on various organs and systems. Chemical and physical methods are devised for the extraction and identification of therapeutic agents and their degradation products from biological systems. The methods used include differential extraction and precipitation, chromatography, visible and ultraviolet spectrophotometry.

Major Findings:

The antibiotic, Puromycin, has been shown to be bound to plasma proteins to only a limited extent. Using a technique involving dialysis and ultraviolet spectrophotometry, it was demonstrated that the portion bound by the albumin fraction could account almost entirely for the total 5 to 7 per cent of the drug so associated over a wide concentration range. Such a phenomenon, therefore, could not adequately explain the apparent disappearance of the compound from the blood of experimental animals.

A total of 20 acid salts of the anti-malarial compound, Daraprim, have been prepared. Solubility determinations in water and chemical analyses have been completed on 7 of these salts.

An investigation of the central nervous system (CNS) effects of the anti-histaminic agent, diphenhydramine (Benadryl), in mice demonstrated an ability of the compound to potentiate the CNS effects of a depressant drug and of a stimulant drug. At a single dosage level which by itself showed no gross effect, diphenhydramine prolonged the sleeping time of pentobarbital and increased

9. (continued)

the convulsive activity of strychnine, the latter being drugs which have long been recognized as pharmacological antagonists.

The following substances extracted with organic amines (N-butyl, diethyl-, triethylamine, and pyridine) were toxic when fed to chicks: soy bean oil meal, cotton seed meal, black-eyed peas; soy protein (but not zein, casein or gelatin); glucose (but not starch or sucrose). On refluxing with triethylamine, glucose, and fructose browned, but not starch, sucrose or dulcitol. A free aldehyde or ketone is essential for browning and browning may be associated with toxicity of amine reacted sugars. Guinea pigs behave similarly to chicks when fed soy meal extracted with organic amines.

Significance to Microbiological Research:

Information contributed by studies in chemical pharmacology are of prime importance towards development of rational approaches to problems in chemotherapy. Such information is derived from examination of drug metabolism and activity in parasite as well as in host and on the interrelationships resulting from the infection of one by the other. The elucidation of modes of drug action by such means are essential in the replacement of present empirical methods. Equally important to the field are the data obtained from animal experimentation on the toxicological and pharmacological properties of potential therapeutic compounds. Evaluation of such information is a prerequisite for the design of safe and effective clinical trials.

Proposed Course of Project:

This project will be continued with emphasis on the toxicology, method of assay, and pharmacodynamics of Puromycin and related compounds.

Project Subtitle:

Experimental Chemotherapy

Objectives:

To discover new and more effective therapeutic agents for the treatment of amebiasis and schistosomiasis.

Methods:

a. Guinea pigs are inoculated with a virulent, test-tube-grown strain of Endamoeba histolytica and then treated. Test compounds are evaluated at non-toxic doses for their ability to prevent death or injury to the host by the parasite.

9. (continued)

b. Mice are infected with known numbers of Schistosoma mansoni cercariae. Treatment is begun about the time worms are sexually mature. Evaluation of test compounds is based on the density of eggs in the liver, and the abundance of dead and live worms as compared to the same factors in untreated mice and those treated with a standard drug.

Major Findings:

a. Thirty-eight analogues of Puromycin were tested and found to be inactive or less active than the parent compound against Endamoeba histolytica. Two antibiotics, an unidentified one from Lederle Laboratories and Actidione were active as amebicides. Amebae, together with bacteria, could be grown in simplified media, the critical ingredient of which was glucosamine. Amebae grown in such media underwent a permanent loss of virulence unless trypticase was present.

b. No compound or combination of compounds possessed any therapeutic advantage over tartar emetic or antimony trichloride against S. mansoni. Pentavalent antimonials were less toxic but also less active than trivalent antimonials. The latter compared favorably in activity with non-antimonial compounds such as Miracil.

Significance to Microbiological Research:

a. There is an obvious need for a more effective and non-toxic amebicide. This can be discovered both through mass screening of miscellaneous compounds and through the logical exploitation of chemical structures known to have some activity against the parasite. Puromycin appears to be a logical compound for exploitation because of its tripartite chemical structure and its peculiar antibiotic spectrum. In addition, further studies on the virulence or pathogenicity of amoebae are needed both from the practical standpoint of maintaining an adequate test system for therapeutic studies and from a basic viewpoint of answering fundamental questions concerning the carrier state versus active amebiasis.

b. Treatment of schistosomiasis remains hazardous and difficult; the discovery of more effective and safer therapeutic agents is a practical necessity. The rationale of the present program is two-fold: (1) mass screening of chemical compounds and (2) systematic exploitation of modifications of chemicals such as Miracil-D and antimonials.

9. (continued)

Proposed Course of Project:

Further evaluation of compounds for the treatment of amoebiasis and schistosomiasis will be carried out but with the main emphasis along lines of drug action.

Budget Data Sheet

10.

11.

12. BUDGET ACTIVITY:

RESEARCH.

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATION PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957.

Project Subtitle: Chemical Pharmacology and Toxicology. The Laboratory of Biochemistry and Nutrition, NIAMD provided dietary materials and consultation.

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PHS (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS) IDENTIFY SUCH RESEARCH.

None

Honors, Awards, and Publication Sheet

15.

16.

LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

Taylor, D. J.; Bond, H. W., and Sherman, J. F.: Puromycin I. Activity against experimental amebiasis. Antibotics Annual, 1954-1955, 745-750.

Bond, H. W., Sherman, J. F., and Taylor D. J.: Puromycin II. Effects of structural changes on amebacidal activity. Antibiotics Annual, 1954-1955, 751-756.

Sherman, J. F., Taylor, D. J., and Bond, H. W.: Puromycin III. Toxicology and pharmacology. Antibiotics Annual, 1954-1955, 757-765.

Nolan, M. O. and Bond, H. W.: Results of laboratory screening tests of chemical compounds for molluscicidal activity III. Derivatives of abietic acid. Am. J. Trop. Med. & Hyg. 4: 152-155, January, 1955.

Greenberg, J.: Inheritance of certain characteristics by Plasmodium gallinaceum. Trop. Med. & Hyg. News 4: 6-14, February, 1955.

Schmidt, L. H. and Coatney, G. R.: Review of investigations in malaria chemotherapy (U. S. A.) 1946 to 1954. Am. J. Trop. Med. & Hyg. 4: 208-216, March, 1955.

Luttermoser, G. W.: Studies on the chemotherapy of experimental schistosomiasis III. Harvest of Schistosoma mansoni cercariae by forced nocturnal emergence from Australorbis glabratus. J. Parasitol. 41: 201-208, April, 1955.

Coatney, G. R.: The present status of the anti-malarial drug chloroquine, pyrimethamine (Daraprim), and primaquine. Industry and Tropical Health, Second Conference, 116-122, 1955.

Coatney, G. R.: Estado actual de las drogas antipaludicas cloroquina pirimetamina (Daraprim) y primaquina. Bol. Ofic. san. panam., 39: 175-182, August, 1955.

Nadel, E. M., Greenberg, J., Jay, G. E., and Coatney, G. R.: Backcross studies on the genetics of resistance to malaria in mice. Genetics, 40: 620-626, September, 1955.

Taylor, D. J. and Greenberg, J.: Hyperactivity of metachloridine against Plasmodium gallinaceum in chicks maintained on a purified diet. Proc. Soc. Exper. Biol. & Med. 90: 551-554, November, 1955.

16. (continued)

In Press

Greenberg, J., Taylor, D. J., and Bond, H. W.: Simple aliphatic substrates in the culture of Endamoeba histolytica. Am. J. Trop. Med. & Hyg.

Greenberg, J., Taylor, D. J., and Bond, H. W.: Glucosamine in the culture of Entamoeba histolytica with a mixed bacterial flora. Am. J. Trop. Med. & Hyg.

Greenberg, J. and Bond, H. W.: Further studies on cross-resistance between pyrimethamine and related compounds. Am. J. Trop. Med. & Hyg.

Greenberg, J.: Differences in the course of Plasmodium berghei infections in some hybrid and backcross mice. Am. J. Trop. Med & Hyg.

Taylor, D. J., Greenberg, J., Josephson, E. S., and Nadel, E. M.: Histochemical sudanophilia and cholesterol concentration in the adrenal glands of chicks parasitized with Plasmodium gallinaceum. Endocrinology.

Sherman, J. F.: The binding of puromycin by plasma proteins. Antibiotics and Chemotherapy.

Luttermoser, G. W., Bond, H. W., and Sherman, J. F.: Studies on the chemotherapy of experimental schistosomiasis IV. Oral activity of antimony trichloride antibiotic complexes. Proc. Soc. Exper. Biol. & Therap.

Greenberg, J.: Mixed lethal strains of Plasmodium gallinaceum: drug-sensitive, transferable (SP) x drug-resistant non-transferable (BI). Exper. Parasitol.

17. HONORS OR AWARDS FOR THE PROJECT

Dr. Howard Bond

President of the Washington Section of the American Chemical Society.
A member of the Council American Chemical Society.

Dr. G. Robert Coatney

Re-appointed as a member of the Panel on Malaria of the subcommittee on Infectious Diseases and Chemotherapy, Committee on Medicine and Surgery, National Research Council.
Appointed visiting lecturer in Tropical Public Health, Harvard School of Public Health.
Appointed visiting professor in the Department of Preventive Medicine and Public Health, School of Medicine, Harvard University.

R.P.C. - 1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

1. National Microbiological Institute
INSTITUTE

2. Rocky Mountain Laboratory
LABORATORY OR BRANCH

3. Office of the Chief
SECTION

4. Hamilton, Montana
LOCATION

5. NMI - 64
SERIAL NO.

6. None

7. Dr. Carl L. Larson
PRINCIPAL INVESTIGATOR

8. None
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives:

The over-all direction and supervision of the scientific research program of the Rocky Mountain Laboratory; the direction of the administrative aspects of the Laboratory including all fiscal, supply, housekeeping and maintenance activities; and the providing of scientific services as required by the research staff including laboratory animal care and breeding, sterile glassware and media preparation, special shop services, library, graphic arts, etc.

R.P.C. - 2
December 1955

Analysis of NIH Program Activities

SERIAL NO. NMI-64

Budget Data Sheet

10.

11.

12. BUDGET ACTIVITY:

RESEARCH

13. None

14. None

R.P.C. - 3
December 1955

Analysis of NIH Program Activities

SERIAL NO. NMI-64

Honors, Awards, and Publications Sheet

15.

16. None

17. None

R.P.C. - 1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

1. National Microbiological Institute
INSTITUTE

2. Rocky Mountain Laboratory
LABORATORY OR BRANCH

3. None

4. Hamilton, Montana
LOCATION

5. NMI 65
SERIAL NO.

6. Transmission of Disease Agents by Various Arthropods
PROJECT TITLE

7. Dr. Cornelius B. Philip
PRINCIPAL INVESTIGATOR

8. Dr. Gordon E. Davis, Dr. Willy Burgdorfer, Dr. David B. Lackman, Dr. J. Frederick Bell, Dr. W. J. Hadlow, Dr. James M. Brennan, and Glen M. Kohls
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives:

The purposes of this study are to inquire into the nature of the relations between certain arthropods and pathogenic agents. These determine the relative efficiency of the vectors, their potential in maintaining infection in nature and their ability to transmit disease to man or other important hosts. These data serve to suggest and implement methods of control of such insect-borne diseases.

Methods Employed:

The methods employed are complex and must be adapted to the variables of vector, host, and pathogenic agent. Colonies of ticks, mites, fleas, and mosquitoes are being reared under laboratory conditions and procedures are instituted to control and prevent loss of the insects involved. A modern insectary is available for these studies. Appropriate species of infectious agents are also maintained in hosts, vectors, and upon suitable culture media. As occasion arises, incidental taxonomic studies concern the parasitic acarina, biting flies, and fleas which may be potential or experimental vectors of such agents.

Major Findings:

Certain species of hard and soft ticks do not become infected with rabies virus when fed on infected hosts. When fed through membranes on blood-brain mixtures and when injected directly, ticks picked up the virus, but this diminished and disappeared within 2 weeks or at time of molting. Leptospira pomona persists and multiplies in both argasid and ixodid ticks. In Ornithodoros turicata, leptospirae persist for 500 days, and 13 of 44 transmission tests resulted positively. No transovarial passage occurred except in one test of Dermacentor andersoni. New data on the biology of relapsing fever tick vectors and a new species of spirochete from Mexico are being reported. O. lahorensis from Turkey repeatedly transmitted Q fever to laboratory animals by bite. The agent of salmon poisoning, which does not grow in chick embryos, was grown in dog node tissue cultures for 8 days without gross cytopathogenic changes nor with reduction in virulence for dogs. Transmission of yellow fever was accomplished by colonies of Aedes aegypti from Australia, Malaya, and Formosa with apparent equal facility. Mosquitoes of A. pseudoscutellaris complex from Southwest Pacific Islands also proved to be efficient vectors. Studies of species of relapsing fever spirochetes by complement fixation showed no consistent species-specific activity. Tests of Amblyomma americanum from the Bullis fever area in Texas again resulted in recovery of strains of Q fever, some of them atypical but serologically positive.

Significance to Microbiological Research:

Both hard and soft ticks may be concerned in transmission of leptospirosis of animals, indicating need for attention to this factor in control. Q fever in parts of the Middle and Far East could be the result of bites on persons due to O. lahorensis. Outside investigators from Johns Hopkins, Tulane, and the Army used Rocky Mountain Laboratory insectary facilities for extended periods in studies on mosquito transmission of western equine and Murray Valley encephalitis with new findings in vector relationships which will increase over-all understanding of the epidemiology of these diseases.

Failure of penetration of the Orient by yellow fever appears unlikely to be due to a mosquito vector barrier. Ticks do not appear to be a likely carrier of rabies in nature. The further elucidation of the involved cycle in nature of the rickettsialike agent of salmon poisoning has created wide-spread interest and an alertness to the potentiality of endoparasites as vectors of disease agents.

Proposed Course of Project:

Studies on tick transmission of tularemia and rabies have been discontinued with final reports in preparation or submitted for publication. A few tests remain to terminate the plague and salmon poisoning studies. Study of the etiologic relationships and experimental transmission of newly acquired strains of Siberian tick typhus are planned, plus attempts to prepare a vaccine. Studies under an outside contract have taken a direction that requires classification. Study of mosquito transmission of St. Louis encephalitis in the insectary will again be pursued by a Johns Hopkins scientist next summer. In connection with the new "trivittatus virus," attempts are being made to colonize *Aedes trivittatus* for transmission studies. Renewed attempts are also under way to analyze factors in the tick transmission of Colorado tick fever virus.

Analysis of NIH Program Activities

SERIAL NO. NMI 65

Budget Data Sheet

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12. BUDGET ACTIVITY:

RESEARCH

13. None

14. None

R.P.C. - 3
December 1955

Analysis of NIH Program Activities

SERIAL NO. NMI 65

Honors, Awards, and Publications Sheet

15.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

1. Philip, Cornelius B.: The types of *Chrysops laeta* Fabricius and a new species of neotropical deerfly in the University of Copenhagen Museum. Ent. Medd., Copenhagen, XXVII, 70-75. 1955.

2. Davis, Gordon E.: Observations of the biology of the argasid tick *Ornithodoros puertoricensis* Fox. J. Parasitol. 41: 76-79. Feb. 1955.

3. Philip, Cornelius B.: El macho de *Bolbodimyia dampfi* Philip (Diptera, Tabanidae). Rev. del Inst. de Salubridad y Enf. Trop. XIV(2): 89-90. June 1954.

4. Davis, Gordon E.: The endemic relapsing fevers. Diseases Transmitted from Animals to Man, 4th ed., Chapt. XXVIII. 1955.

5. Philip, Cornelius B.: The horse-flies (Diptera: Tabanidae) of the Ethiopian Region. Vol. II: *Tabanus* and Related Genera, written by H. Oldroyd. Science 121(3132): 27. Jan. 7, 1955.

6. Davis, Gordon E., and Mavros, Anthony J.: The long survival of *Borrelia hispanica* (de Buen) in the argasid tick *Ornithodoros nicollei* Mooser. A problem in xenodiagnosis. Exp. Parasitol. 4(3): 277-281. May 1955.

7. Davis, Gordon E., and Burgdorfer, Willy: Relapsing fever spirochetes: An aberrant strain of *Borrelia parkeri* from Oregon. Exp. Parasitol. 4(2): 100-106. March 1955.

8. Burgdorfer, W.: On the "occult" infection in relapsing fevers. Bull. Soc. Path. exot. 47(5): 664-667. Sept.-Oct. 1954.

9. Locker, Betty: The identification of *Taenia tenuicollis* Rudolphi, 1819, in North America. J. Parasitol. 41(1): 51-56. Feb. 1955.

10. Philip, Cornelius B.: New North American Tabanidae. IX. Notes on and keys to the genus *Chrysops* Meigen. Rev. Brasil. Ent. 3: 47-128. June 1955.

11. Philip, Cornelius B.: Historic material of the biting fly Family Tabanidae from the New World in European Museums. Amer. Phil. Soc. Year Book 1954, pp. 147-148.

12. Philip, Cornelius B., and White, J. S.: Disease agents recovered incidental to a tick survey of the Mississippi Gulf Coast. J. Econ. Ent. 48(1): 396-400. Aug. 1955.

13. Philip, Cornelius B.: Is the association of organisms with arthropod tissues a necessary concept in defining the Family Rickettsiaceae? VI Cong. Intern. di Microbiol., Rome, 6-12 Sept. 1953, 5: Sez. XVI, pp. 522-528.

14. Philip, Cornelius B., and Hughes, Lyndahl E.: Disease agents found in the rabbit tick, Dermacentor parumapertus, in the southwestern United States. VI Cong. Intern. di Microbiol., Rome, 6-12 Sept. 1953, 5: Sez. XVI, pp. 541-548.

15. Philip, Cornelius B.: There's always something new under the "parasitological" sun. J. Parasitol. 41(2): 125-148. April 1955.

16. Philip, Cornelius B., Hadlow, W. J., and Hughes, L. E.: Neorickettsia helmintheca, a new rickettsialike disease agent of dogs in western United States transmitted by a helminth. VI Cong. Intern. di Microbiol., Rome, 6-12 Sept. 1953, 4: Sez. XI, pp. 70-82.

17. Philip, Cornelius B., Bell, J. Frederick, and Larson, Carl L.: Evidence of infectious diseases and parasites in a peak population of black-tailed jack rabbits in Nevada. J. Wildlife Management 19: 225-233. April 1955.

18. Davis, Gordon E.: Relapsing fever spirochetes: The present status of Borrelia venezuelensis Brumpt and Borrelia neotropicalis Bates and St. John. Intern. Bull. Bact. Nomenclature & Taxonomy 5(3): 107-109. July 15, 1955.

19. Davis, Gordon E.: Letter to the editor. Amer. J. Trop. Med. & Hyg. 4(5): 961-962. Sept. 1955.

20. Kohls, Glen M., and Locker, Betty: Isolation of Pasteurella tularensis from the tick Ixodes ricinus in Norway. Nordisk Vet. 6: 883-884. 1954.

21. Jellison, W. L., and Kohls, Glen M.: Tularemia in sheep and in sheep-industry workers. Pub. Health Monograph No. 28, 17 pp. 1955.

22. Kohls, Glen M.: Two new species of ticks from North Borneo (Acarina: Ixodidae). J. Parasitol. 41(3): 312-315. June 1955.

23. Brennan, James M.: Chatia Brennan, 1946: A new generic character and additional notes (Acarina: Trombiculidae). J. Parasitol. 41(3): 283-284. June 1955.

24. Hadlow, W. J., Grimes, E. F., and Jay, G. E., Jr.: Stilbestrol-contaminated feed and reproductive disturbances in mice. Science 122: 643-644. Oct. 7, 1955.

In Press

1. Brennan, James M., and Beck, D. Elden: The chiggers of Utah (Acarina: Trombiculidae). Great Basin Nat.

2. Burgdorfer, Willy, and Owen, Cora Rust: Experimental studies on argasid ticks as possible vectors of tularemia. J. Inf. Dis.

3. Davis, Gordon E.: The identification of spirochetes from human cases of relapsing fever by xenodiagnosis with comments on local specificity of tick vectors. Exp. Parasitol.

4. Davis, Gordon E.: A relapsing fever spirochete, Borrelia mazzottii, n.sp., from Ornithodoros talaje from Mexico. Amer. J. Hyg.

5. Davis, Gordon E.: World distribution of spirochetal diseases. 2. Relapsing fevers, louse-borne and tick-borne. (Amer. Geog. Soc., plate 16). Amer. J. Trop. Med. & Hyg.

6. Davis, Gordon E., and Mavros, Anthony J.: An atypical Ornithodoros hermsi from Utah (Ixodoidea: Argasidae). J. Parasitol.

7. Davis, Gordon E., and Mavros, Anthony J.: Observations on the biology of Ornithodoros delanoei Roubaud and Colas-Belcour, 1931 (Ixodoidea: Argasidae). Bull. Soc. Path. exot.

8. Davis, Gordon E., and Mavros, Anthony J.: Observations on the biology of Argas brumpti Neumann, 1907 (Ixodoidea: Argasidae). Bull. Soc. Ent. d'Egypte.

9. Davis, Gordon E., and Hoogstraal, Harry: Studies on the biology of the relapsing fever spirochete, Borrelia persica, recovered from the argasid tick, Ornithodoros tholazani, collected in the Western Desert Governorate, Egypt; with comments on the distribution and ecology of the tick vector. Ann. de Parasitol.

10. Kohls, Glen M.: Concerning the identity of Amblyomma maculatum, A. tigrinum, A. triste, and A. ovatum of Koch 1844 (Acarina: Ixodidae). Proc. Ent. Soc. Wash.

11. Kohls, Glen M.: The identity of Ixodes boliviensis Neumann 1904 and I. bicornis Neumann 1906 (Ixodidea). Proc. Ent. Soc. Wash.

12. Kohls, Glen M.: Ticks (Ixodoidea) of Borneo and Malaya. Malaysian Parasites. Inst. Med. Res., Kuala Lumpur, Malaya.

13. Philip, Cornelius B.: Comments on the classification of the Order Rickettsiales. Amer. J. Microbiol.

14. Philip, C. B., and Fairchild, G. B.: American biting flies related to Chlorotabanus Lutz and Criptolylus Lutz (Diptera: Tabanidae). Ann. Ent. Soc. Amer.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

Philip, Cornelius B.

Invited to give two lectures at Ohio State University, Columbus, Ohio, June 9, 1955.

Elected Chairman of Section D, Medical and Veterinary Entomology, of the American Entomological Society.

Invited to contribute a paper on rickettsial agents for a Russian medical journal, the first invitation from the U.S.S.R. since the War to an American to contribute a medical paper.

Davis, Gordon E.

Invited by the editor of the American Journal of Tropical Medicine & Hygiene to prepare a critical review of the section on the relapsing fevers published by the American Geographical Society.

R.P.C. - 1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

1. National Microbiological Institute
INSTITUTE

2. Rocky Mountain Laboratory
LABORATORY OR BRANCH

3. None

4. Hamilton, Montana
LOCATION

5. NMI 66
SERIAL NO.

6. Studies on Diseases Having a Reservoir of Infection in the Natural Environment
PROJECT TITLE

7. Dr. J. Frederick Bell
PRINCIPAL INVESTIGATOR

8. Dr. David B. Lackman, Dr. William L. Jellison, Dr. William J. Hadlow, Dr. Cora R. Owen, Dr. Lauri Luoto, and Dr. Carl L. Larson.
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives:

The objectives of this project are to determine the extent of certain diseases as psittacosis, rabies, and coccidioidomycosis in the United States and to discover methods for the control of certain of these.

Methods Employed:

The methods are variable depending upon the objectives. These include field studies as in the case of coccidioidomycosis and psittacosis and specialized laboratory approaches as in the case of Q fever.

Major Findings:

Rabies has been detected in bats on 3 further occasions in addition to the single isolation recorded last year. It is significant that among 140 bats collected by intent (and presumed to be healthy) no isolations were made while isolations were made from each of 3 bats found sick by cooperative laymen.

Two isolations of Coccidioides immitis were made from rodents in Utah, and Haplosporangium parvum has been isolated from lung tissue of animals from Sweden.

A continuation of the Q fever vaccine program indicates that the incidence of Q fever among vaccinated cattle is decreased by 93% as compared to control animals contained in infected herds. This immunity persists for at least 3 years. The capillary-tube test devised for study of milk specimens has been applied with excellent results to serums from both men and animals, and the test has been applied successfully by investigators cooperating through the agency of WHO.

Genetic studies of Clostridium botulinum, type C, have yielded a stable toxigenic clone, and it has been demonstrated that these organisms produce their effects in nature through elaboration of toxin in animal matter existing as a microenvironment rather than in decaying plant matter as was previously held by others.

Reliable criteria for establishing the virulence of Bacterium tularense were developed.

The presence of psittacosis virus or antibodies was demonstrated in 46 patients and 23 parakeets. The presence of bovine encephalitis in Montana, Utah, and Ohio was established by serological methods.

Significance to Microbiological Research:

The demonstration of induced immunity in cattle against infections with Coxiella burnetii points to a practical method of control of Q fever in the animal reservoir and thus in man. The capillary-tube test for the diagnosis of this disease presents a practical, simple, and inexpensive method of survey to determine the need for institution of control methods. The isolation of toxigenic clones from Cl. botulinum, type C, indicates that the genetic characteristics of these organisms are stable. The relation of rabies infection in bats to the maintenance and spread of this infection among other animals must be further studied since it may have certain relations to outbreaks of disease in local areas. It is significant that in Montana no rabies has been reported in animals other than bats for the last 6 years.

Proposed Course of Project:

No major emphasis will be given this project in the forthcoming year. The Q fever vaccine program will be terminated, and studies of rabies and coccidioidomycosis will be pursued only as time permits.

R.P.C. - 2
December 1955

Analysis of NIH Program Activities

SERIAL NO. NMI

Budget Data Sheet

10.

11.

12. BUDGET ACTIVITY:

RESEARCH

13. None

14. None

Honors, Awards, and Publications Sheet

15.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

1. Hadlow, W. J., and Stoenner, H. G.: Histopathological findings in cows naturally infected with *Leptospira pomona*. Am. J. Vet. Res. *16*: 45-56. Jan. 1955.

2. Jellison, W. L., and Reinhard, Karl: Leptospirosis. Diseases Transmitted from Animals to Man, 4th Ed., Chapt. XXVI. 1955.

3. Jellison, W. L.: Rat-bite Fever. Diseases Transmitted from Animals to Man, 4th Ed., Chapt. XXVII. 1955.

4. Jellison, W. L., Geary, John M., Harkema, R., and Ross, N.C.: An outbreak of schistosome dermatitis in Alaska. 3d Alaska Sci. Conf. pp. 153-154. Sept. 1954.

5. Bell, J. Frederick, Sciple, George W., and Hubert, A. A.: A microenvironment concept of the epizoology of avian botulism. J. Wildlife Management *19*(3): 352-357. July 1955.

6. Ormsbee, Richard A., Bell, J. Frederick, and Larson, Carl L.: Studies of *Bacterium tularense* antigens. I. The isolation, purification, and biologic activity of antigen preparations from *Bacterium tularense*. J. Immunol. *74*(5): 351-358. May 1955.

7. Ormsbee, Richard A., and Larson, Carl L.: Studies on *Bacterium tularense* antigens. II. Chemical and physical characteristics of protective antigen preparations. J. Immunol. *74*(5): 359-370. May 1955.

8. Larson, Carl L.: Rocky Mountain spotted fever. Am. J. Nursing *55*: 716-719. June 1955.

9. Luoto, Lauri, and Mason, Donald M.: An agglutination test for bovine Q fever performed on milk samples. J. Immunol. *74*(3): 222-227. March 1955.

10. Ormsbee, Richard A.: Soluble antigen and antibiotic resistance as factors in the classification of *Coxiella burnetii*. VI Intern. Cong. di Microbiol., Rome, 6-12 Sept. 1953. Vol. 5, Sez. XVI, pp. 15-20.

11. Ribi, Edgar, and Shepard, C. C.: Morphology of Bacterium tularense during the growth cycle in liquid medium as revealed by the electron microscope. Exp. Cell Res. (Sweden) 8(3): 474-487. June 1955.

12. Stoenner, Herbert G.: The management of herds affected by bovine leptospirosis. "Proceedings Book" A.V.M.A. 91st annual meeting, Seattle, Aug. 23-26, 1954, pp. 83-87. Aug. 1955.

13. Ormsbee, Richard A., Parker, Hazel, and Pickens, Edgar G.: The comparative effectiveness of aureomycin, terramycin, chloramphenicol, erythromycin, and thiocymetin in suppressing experimental rickettsial infections in chick embryos. J. Inf. Dis. 96(2): 162-167. Mar.-Apr. 1955.

14. Larson, Carl L., Wicht, William, and Jellison, W. L.: A new organism resembling P. tularensis isolated from water. Pub. Health Rep. 70(3): 253-258. March 1955.

15. Hadlow, W. J.: Degenerative myopathy in a white-tailed deer, Odocoileus virginianus. Cornell Vet. 45(4): 538-547. Oct. 1955.

16. Bell, J. Frederick, Hadlow, W. J., and Jellison, W. L.: Chiropteran rabies in Montana. Pub. Health Rep. 70(10): 991-994. Oct. 1955.

17. Bell, J. Frederick, Owen, Cora R., and Larson, Carl L.: Virulence of Bacterium tularense. I. A study of the virulence of Bacterium tularense in mice, guinea pigs, and rabbits. J. Inf. Dis. 97(2): 162-166. Sept.-Oct. 1955.

18. Owen, Cora R., Bell, J. Frederick, Larson, Carl L., and Ormsbee, Richard A.: Virulence of Bacterium tularense. II. Evaluation of criteria of virulence of Bacterium tularense. J. Inf. Dis. 97(2): 167-176. Sept.-Oct. 1955.

19. Shepard, Charles C., Ribi, Edgar, and Larson, Carl L.: Electron microscopically revealed structural elements of Bacterium tularense and their in vitro and in vivo role in immunologic reactions. J. Immunol. 75(1): 7-14. July 1955.

20. McKee, Max T.: Bacterial culture with controlled pH. Applied Microbiol. 3(6): 355-360. Nov. 1955.

In Press:

1. Hadlow, W. J.: Necropsy procedure for ruminants. "Proceedings Book" A.V.M.A. 92nd annual meetings, Minneapolis, 1955.

2. Jellison, William L.: On the nomenclature of *Besnoitia besnoiti*, a protozoan parasite. Proc. N. Y. Acad. Sci.

3. Jellison, William L.: Sodoku. Rat-bite fever due to *Spirillum minus* Carter. Diagnostic Procedures and Reagents, edited by Dr. William B. Cherry.

4. Jellison, William L., Fullerton, W. J., and Parker, Hazel: Transmission of the protozoan, *Besnoitia jellisoni*, by ingestion. Proc. N. Y. Acad. Sci.

5. Adams, Lowell, Salvin, S. B., and Hadlow, W. J.: Ringworm in a population of snowshoe hares. J. Mammal.

6. Stoenner, Herbert G.: Application of serology to the diagnosis of leptospirosis. "Proceedings Book" A.V.M.A., 92d annual meetings, Minneapolis, 1955.

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17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

Hadlow, W. J.

Invited to give a demonstration of necropsy procedures at the meeting of the American Veterinary Medical Association in Minneapolis, Aug. 15-18, 1955.

Jellison, William L.

Invited to give lecture on epidemic hemorrhagic fever for students in Preventive Medicine Orientation course, Fort Sam Houston, Texas, May 31, 1955.

R.P.C. - 1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

1. National Microbiological Institute
INSTITUTE

2. Rocky Mountain Laboratory
LABORATORY OR BRANCH

3. None

4. Hamilton, Montana
LOCATION

5. NMI 67
SERIAL NO.

6. Study of Diseases Transmitted by Arthropods with Particular Emphasis on the Following Virus Diseases: Colorado Tick Fever, the Western Equine Type of Encephalitis, and St. Louis Encephalitis
PROJECT TITLE

7. Dr. Carl M. Eklund
PRINCIPAL INVESTIGATOR

8. Glen M. Kohls, Dr. James M. Brennan, Dr. William L. Jellison, Dr. David Lackman, and Dr. William J. Hadlow
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

A. Colorado tick fever

Objectives:

Colorado tick fever has been regarded as a relatively benign disease following tick bite as contrasted with Rocky Mountain spotted fever. The disease has been recognized and studied previously in Colorado and little was known about its frequency elsewhere. Following the unexpected discovery of virus-carrying ticks and clinical disease in the Bitterroot Valley, studies were initiated to determine the geographic distribution of virus-carrying ticks and of human infection. Following the discovery in children of severe illness with symptoms of encephalitis it became evident that a study of the clinical picture produced by infection with CTF virus is necessary. To understand the natural history of the virus a greater knowledge of the geographic distribution of infected ticks is needed.

In summary, the objectives are: determine the importance of CTF as a public health problem, determine the important tick species which transmit the virus to man, determine the ecological features necessary to maintain virus-infected ticks, devise and evaluate methods of diagnosis, and define the clinical picture more clearly.

Methods Employed:

All specimens of blood sent in to the laboratory from patients with a history of tick exposure were examined for the presence of CTF virus and for neutralizing antibodies regardless of the type of clinical disease reported.

Collections of ticks were made by laboratory personnel for examination for the presence of virus. These collections from western areas were supplemented by small numbers of ticks sent in by persons living in other parts of the country.

Columbian ground squirrels were collected; larval and nymphal ticks obtained from them were examined for virus and the ability of these mammals to infect ticks following injection of virus studied.

Ticks found infected in nature and ticks infected in the laboratory were studied to determine whether transovarial transmission of the virus took place.

Major Findings:

Clinical disease has been detected in most western states and as far east as western South Dakota. Serious illness with symptoms of encephalitis has been found in children. Last summer a 4-year-old child died following an illness characterized by symptoms of encephalitis and purpuric manifestations. There was a history of the removal of a tick a few days prior to illness; CTF virus was isolated. The disease corresponds to the seasonal distribution of *D. andersoni* ticks. The spring of 1955 was very late and the early summer cold; ticks appeared late and persisted throughout the summer; the disease followed the same pattern.

D. andersoni is the only tick species commonly feeding on man within the distribution of the human disease from which virus has been isolated. Virus has been isolated from other tick species (*D. parumapertus*, *D. occidentalis*, *O. lagophilus*) but the significance of this finding is not clear at present. Human disease has been acquired only within the geographic distribution of *D. andersoni*. In every area within the distribution of *D. andersoni* where it has been possible to do reasonably adequate collecting, virus has been found. This year virus was isolated for the first time from ticks (*D. andersoni*) collected in South Dakota (Black Hills), northern New Mexico, and Utah, thus extending considerably the known geographical distribution of virus-carrying ticks. Virus was frequently isolated from *D. andersoni* collected in Oregon, Washington, Idaho, and western Montana.

Virus has been isolated from D. andersoni nymphs collected from Columbian ground squirrels. It has been possible to infect Columbian ground squirrels and to infect nymphs feeding on them.

Transovarial transmission of virus could not be demonstrated using ticks infected in nature or ticks infected in the laboratory. It appears that CTF virus is maintained by a cycle of infection invading small mammals and larval and nymphal ticks.

D. variabilis, a species closely related to D. andersoni, has never been found infected in nature even when collected in areas where D. andersoni is found. Virus was not isolated from numerous D. variabilis from eastern Montana, southwestern South Dakota, and western Nebraska, although several isolations of Pasteurella tularensis were made from these ticks. Tests of numerous Amblyomma americanum from Camp Bullis, Texas, were negative for CTF virus. Some of these latter ticks proved to be infected with the Q fever organism.

Studies of pathology in mice infected with CTF virus demonstrate constant cerebellar and myocardial lesions.

Only 15 of 73 human cases of CTF from whom virus was isolated gave positive complement-fixation tests with this virus, demonstrating the relative inadequacy of serological tests for the diagnosis of this disease.

Significance to Microbiological Research:

The most important finding of the present study has been that without an interested group of investigators and the provision of diagnostic methods, a frequent and important disease can be largely overlooked, hidden under a variety of clinical diagnoses. It has been learned that Colorado tick fever occurs frequently every spring and summer (isolation of virus from 75-85 patients each year plus others diagnosed by neutralization tests) and is to be expected throughout the range of D. andersoni, including the most attractive vacation areas in the West.

A tick species has been shown to be one of the most efficient of arthropods in the maintenance of a virus, at least the equal of any other known mechanism for maintaining a virus. It is far more efficient then mosquitoes where virus appears in an erratic fashion.

Proposed Course of Project:

The clinical picture must be considered as incompletely known. Now that physicians have become interested in the disease, an effort should be made to determine the extent of serious disease and its clinical features.

The geographic distribution of disease and of infected ticks has been determined in only a rough fashion and many areas within *D. andersoni* distribution need exploring.

The natural history of the CTF virus is chiefly conjecture to date; certain tick species are known to harbor virus. Columbian ground squirrels have viremia, and virus was isolated from *D. andersoni* nymphs on one occasion. An intensive study of small mammals and larval and nymphal ticks collected from them is needed to firmly establish the mechanism by which the virus is maintained.

Transovarial transmission of virus is said to play an important role in the maintenance of Russian spring-summer encephalitis virus. It appears strange that this should be so when such a mechanism is lacking in the case of CTF virus. To avoid dangers involved in handling the RSSE virus, work has been started using louping ill virus. *D. andersoni* nymphs have been infected; when they have moulted to adults they will be fed on a sheep, and progeny examined for virus. Later work with RSSE virus will be done.

In nature, mosquito and tick viruses seem to stay within their respective arthropod hosts. Laboratory work will be done to determine whether mosquito virus such as the St. Louis and Western equine viruses can be readily adapted to ticks. The reverse procedure will also be tried in an attempt to determine why ticks and mosquitoes do not exchange viruses.

B. Western Equine and St. Louis Encephalitides; Other Viruses Associated with Mosquitoes

(The epidemic occurrence of the mosquito-transmitted encephalitides is an infrequent and unpredictable event. Because of this, such epidemics cause much more disturbance than do the more familiar diseases. The infrequent occurrence over extensive areas, often sparsely populated, does not justify a continuous mosquito control and/or vaccine program. Effective control appears possible only when knowledge of the natural history of these viruses is sufficient to enable one to predict the occurrence of an epidemic.)

Objectives:

Correlate human disease with mosquito abundance as well as with incidence of infected mosquitoes, study ecology of these viruses, determine mechanism for survival of these viruses; from the above establish base lines for predicting epidemics, develop effective and practical methods of control, and characterize and determine importance of unknown viruses harbored by mosquitoes.

Methods Employed:

Field Studies. In selected areas (western equine in Bismarck, North Dakota; St. Louis and western equine in Council, Idaho) collect mosquitoes and observe occurrence of human disease over a long period in order that a correlation may be established between a virus-carrying mosquito species and the appearance of human cases. Two types of areas are represented--an irrigated area and a northern plains area. By observation of mosquitoes and birds during spring and early summer, determine the relative role of hibernating mosquitoes and birds in carrying virus through the winter months.

Laboratory Studies. Vectors incriminated by field studies are adapted to laboratory conditions, then the minimal amount of virus necessary to infect is determined. The extrinsic incubation, the length of time a mosquito remains infected, the number of times it can transmit, the amount of virus transmitted are all studied under conditions as closely similar to those found in nature as can be arranged. Hibernation is produced under conditions simulating those found in nature, and winter carry-over in such mosquitoes is studied.

Major Findings:

Western equine. Both in California (1952) and in North Dakota (1953) the occurrence of epidemic human disease was preceded by the appearance of enormous numbers of *Culex tarsalis* and a high infection rate in mosquitoes (ca 1%). During seasons with a low incidence of human disease, only *C. tarsalis* has consistently been found carrying virus. It is concluded that it is the only important vector of the western equine virus. Hibernating infected *C. tarsalis* have been kept alive in a root cellar until March. *C. tarsalis* needs a blood meal with a titer of at least 10^4 before appreciable numbers become infected. The shortest extrinsic incubation period is 4 days; the average about 7 days. Virus is carried throughout life (60+ days). A single mosquito may transmit several times. Growth of virus is found only in the mid gut and salivary glands.

St. Louis encephalitis - North Dakota. In an 8-year observation period, St. Louis virus was isolated from *C. tarsalis* during only one season--1954. The incidence of people with St. Louis antibodies is very low (ca 1%). Human disease is very rare. It appears that in contrast with the western equine virus, the St. Louis virus is not effectively maintained in areas with short summers and long, cold winters. C. tarsalis appears to be a vector, but not a particularly efficient one.

Idaho. The incidence of St. Louis antibodies is high--40% in the Council area in two separate surveys. St. Louis encephalitis, however, has not been recognized. Mosquitoes were collected for the first time during the summer of 1955 and three isolations of St. Louis virus from C. tarsalis were made. These strains appear somewhat different from the standard Hubbard strain that was isolated from a patient in St. Louis.

Nevada. In connection with this finding, it is of interest that a small outbreak of about a dozen cases of St. Louis encephalitis studied in Las Vegas, Nevada, during September 1955 appeared to be clinically mild in contrast to cases reported from St. Louis in 1933 and 1937. This mild disease has also been reported from California in previous years. There the explanation was good treatment. This appears questionable. A different strain of St. Louis virus appears more plausible. Although an adequate sample of mosquitoes was collected in Nevada, no virus was isolated. This, in the face of an epidemic, is very puzzling.

Laboratory work. Study of viremia in birds and transmission by C. tarsalis has only started.

Trivittatus and inornata viruses. A virus related to the California virus of Hammon and Reeves has been isolated on a number of occasions from Aedes trivittatus mosquitoes collected near Bismarck, North Dakota. Antibodies have been found in the sera of human beings and horses. Human disease due to the virus has not been established as yet.

During 1952, an agent was isolated from 4 lots of Culex inornata mosquitoes collected near Bismarck. This agent is filterable through a Seitz filter (also Berkefelds) and is not related to common mosquito viruses. Antibody surveys are just starting.

Significance to Microbiological Research:

C. tarsalis has been established as the important vector of western equine encephalitis. The same vector appears to be of importance in the dissemination of the St. Louis virus. The appearance of the western equine type of encephalitis is directly correlated with fluctuation in the numbers of this species and with the incidence of carriers, but is not completely understood. Laboratory studies show that C. tarsalis is an efficient vector of the western equine virus.

As to winter carry-over of the western equine virus, hibernating infected C. tarsalis must be considered a possibility.

Significance to Microbiological Research (Continued)

Although superficially the epidemiology of the western equine and St. Louis encephalitides appears similar, enough data have been accumulated to indicate that the natural history of these viruses must have important differences.

Proposed Course of Project:

Western equine. Chief emphasis is to be laid on the problem of winter carry-over of virus. Work is being continued on the role of hibernating C. tarsalis. Work will be started on the role of migrating birds. They will be trapped and brought into the laboratory to determine whether virus appears in their blood in the absence of mosquito exposure. State health department laboratories are being encouraged to assume the responsibility of establishing the diagnosis in the case of the western equine and St. Louis encephalitides. Antigens and hyperimmune sera are prepared and given to them.

St. Louis virus. In Idaho, how much clinical disease occurs during the acquisition of antibodies by 40% of the population? Is the virus isolated in western areas different in any way from that previously isolated in the St. Louis area? (The high incidence of antibodies, with apparently little serious human disease, indicates that the western strains are different.) We have little data on the variation in numbers of mosquitoes from year to year and the yearly variation in incidence of virus carriers and no correlation of such data with human disease. There is no detailed knowledge on behavior of virus in C. tarsalis, the amount of virus necessary to infect mosquitoes, and the concentration of virus found in the blood of common birds and mammals. The three areas from which we have some knowledge of St. Louis encephalitis are topographically and ecologically different. North Dakota is a natural area representative of the northern plains; C. tarsalis is abundant, but little virus is present. The Idaho study area is an irrigated valley; C. tarsalis is abundant, the incidence of antibodies is very high, virus is present in mosquitoes, but no clinical disease has been recognized. Las Vegas, Nevada is an isolated resort city in a desert, no irrigation or cultivation, C. tarsalis is abundant, a minor epidemic was recognized; no virus in mosquitoes. What are the underlying factors of these very different conditions?

Trivittatus and inornata viruses. Antibody surveys will be conducted on a more extensive scale and more effort devoted to detecting human disease due to these viruses.

Analysis of NIH Program Activities

SERIAL NO. NMI

Budget Data Sheet

10.

11.

12. BUDGET ACTIVITY:

RESEARCH

13. None

14. None

R.P.C. - 3
December 1955

Analysis of NIH Program Activities

SERIAL NO. NMI

Honors, Awards, and Publications Sheet

15.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

1. Eklund, Carl M., Kohls, Glen M., and Brennan, James M.: Distribution of Colorado tick fever and virus-carrying ticks. J.A.M.A. 157(4): 335-337. Jan. 22, 1955.
2. Kohls, Glen M.: Colorado tick fever discovered in California. Calif. Vector Views 2(4): 17. April 1955.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

Kohls, Glen M. Requested to give lecture on Colorado tick fever for students in Preventive Medicine Orientation Course, Fort Sam Houston, Texas, May 31, 1955.

Eklund, Carl M. Elected as a Fellow of the New York Academy of Science.

R.P.C. - 1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

1. National Microbiological Institute
INSTITUTE

2. Rocky Mountain Laboratory
LABORATORY OR BRANCH

3. None

4. Hamilton, Montana
LOCATION

5. NMI
SERIAL NO.

6. Isolation and Characterization of the Polysaccharide Skeleton of the Cell Wall of Histoplasma capsulatum
PROJECT TITLE

7. Dr. Edgar Ribi
PRINCIPAL INVESTIGATOR

8. Dr. Samuel B. Salvin and Dr. Bill H. Hoyer
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

(This project was a continuation of earlier projects on separation of antigens of H. capsulatum.)

Objectives:

It has been shown that the cell wall of H. capsulatum contained the antigenic activity associated with complement fixation, protection, and toxins, whereas the protoplasm was found to be nonantigenic. Attempts were therefore made to study the fine structure of the cell wall during which preliminary studies by means of electron microscopy and x-ray diffraction revealed that the cell wall is made up of a thin outer granular layer and a thick inner fibrillar layer of chitin.

The objective of this project was, primarily, to separate and purify this polysaccharide skeleton and to test it for immunologic properties, and secondarily, to define more closely its submicroscopic morphology and structure.

Methods Employed:

1. Separation of cell wall from protoplasm by methods we have previously described (Proc. Soc. Exp. Biol. & Med. 90: 287, 1955).

2. Isolation of the chitin by degradation of nonchitinous cell-wall constituents with dilute mineral acid.

3. Purification of the chitinous acid residues by subsequent peptization and centrifugation procedures. All steps were controlled by electron microscopy, x-ray diffraction, paper chromatographic protein analysis, and nitrogen and glucosamine determinations.

Major Findings:

The cell wall of *H. capsulatum* contains a network of chitin fibrils with a uniform thickness of about 50 to 100 Å. The chain macromolecules of poly-glucosamine of which the fibrils are made and which run parallel to the length axis of the fibrils are ordered in a crystal lattice over given areas only. Thus, crystalline regions (micelles) with average length of about 100 Å alternate with considerable portions of less well-ordered amorphous-mesomorphous regions. The dimensions of the crystallized areas perpendicular to the main valence chains correspond to the thickness of the fibrils (50 to 100 Å). Purified fungal chitin was found to be nonantigenic. The antigenic properties therefore are expected to be located in the granular outer layer of the cell wall, which is made up of lipid and protein or protein-carbohydrate complex.

Similar results were found during analogous studies of the cell wall of *Blastomyces dermatitidis*, which had been initiated in correlation with studies of *H. capsulatum*.

Significance to Microbiological Research:

The findings shed light on the mechanism of certain cell-wall properties which play a controlling role in the life of the fungal and bacterial cell. They also added to the general knowledge of the nature of the so-called micellar skeleton of the cell walls of plants and, consequently, could be closely correlated with the present concepts of the submicroscopic structure of natural and synthetic fibers.

The micellar system within the chitin fibrils of *H. capsulatum* and *B. dermatitidis* is analogous to that of the submicroscopic fibrils of regenerated cellulose, viscose rayon, which has been extensively studied. Consequently, the physical properties of artificially-made membranes of regenerated cellulose are expected to have properties similar to the chitin skeleton of the cell walls. Rigidity, ductility, and elasticity of the fungal cell wall is well explained by the network arrangement of the chitin fibrils and by the expected flexibility of the fibrils themselves in which the less well-ordered portions act as hinges.

Proposed Course of Project:

This project has been terminated.

Budget Data Sheet

10.

11.

12. BUDGET ACTIVITY:

RESEARCH

13. None

14. None

Analysis of NIH Program Activities SERIAL NO. NMI

Honors, Awards, and Publications Sheet

15.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

1. Salvin, S. B.: Resistance to reinfection in experimental histoplasmosis. J. Immunol. 74(3): 214-221. March 1955.

2. Salvin, S. B.: Further studies on immunization of mice against *Histoplasma capsulatum*. Amer. J. Hyg. 61(1): 72-81. Jan. 1955.

3. Salvin, S. B.: Hypersensitivity in mice with experimental histoplasmosis. J. Immunol. 75(1): 1-6. July 1955.

4. Salvin, S. B., and Bell, E. John: Resistance of mice with experimental histoplasmosis to infection with *Rickettsia typhi*. J. Immunol. 75(1): 57-62. July 1955.

5. Salvin, S. B., and Ribi, Edgar: Antigens from yeast phase of *Histoplasma capsulatum*. II. Immunologic properties of protoplasm vs. cell walls. Proc. Soc. Exp. Biol. & Med. 90(1): 287-294. Oct. 1955.

In Press:

1. Ribi, Edgar, and Salvin, S. B.: Antigens from the yeast phase of *Histoplasma capsulatum*. I. Morphology of the cell as revealed by the electron microscope. Exp. Cell. Res. (Sweden).

2. Salvin, S. B.: An antigen for the detection of precipitins against *Histoplasma capsulatum*. NOTES in J. Bact.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

Salvin, S. B.:

Invited to attend the International Symposium on Therapy of Fungus and Fungus Infections at the University of California Medical School, June 23-25, 1955, and to present results of his studies on the protection of mice against infection with *Histoplasma capsulatum*.

R.P.C. - 1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

1. National Microbiological Institute
INSTITUTE

2. Rocky Mountain Laboratory
LABORATORY OR BRANCH

3. None

4. Hamilton, Montana
LOCATION

5. NMI-69
SERIAL NO.

6. Immune Prophylaxis of Salmonella Infections: Separation and Immunologic Significance of Antigens
PROJECT TITLE

7. Dr. Kelsey C. Milner
PRINCIPAL INVESTIGATOR

8. Dr. Edgar Ribi, and Dr. Bill H. Hoyer
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives:

To isolate, chiefly by physical means, and characterize the active somatic antigens from pathogenic strains of Salmonella; to determine the relationship of such antigens to the "endotoxins" of Boivin and others; and to develop practical vaccines which will be more effective and less toxic than those in current use.

Methods Employed:

A highly virulent strain of Salmonella enteritidis has been used for most studies to date, although the use of other types of Salmonella is contemplated. Flagellae are mechanically removed. Washed cells are agitated in the Mickle apparatus in a manner which cracks the cell walls and releases the protoplasm in a dispersed state. Cell walls and protoplasm are then separated by centrifugation. All steps of mechanical separation are controlled by frequent observation with the electron microscope. Toxic substances are liberated in soluble form into the aqueous phase when water suspensions of cells or cell walls are shaken with ether. The toxin is refined by salt precipitation and dialysis. Nitrogen determinations, x-ray diffraction, paper electrophoresis, and sedimentation studies with the optical ultracentrifuge are performed on certain of the fractions obtained. All fractions are tested serologically as flocculating antigens, as vaccines against the experimental disease in animals, and for toxic effect on mice.

The cell walls contained the antigenic activity associated with protection and toxin and gave high titers in flocculation tests with immune sera. Although toxic substances of the cell walls could be released in soluble form, fully detoxified cell walls have only recently become available for testing as vaccines. Nevertheless, partially detoxified cell walls showed protection superior to that provided by either alcohol-killed intact organisms (Felix-type antigens) or unpurified soluble toxin. These results are an indication that the "endotoxin" is not necessarily identical with the antigen eliciting the formation of protective antibodies, as is generally accepted at present. A conclusion cannot be firmly drawn, however, until fully detoxified cell walls and purified toxin have been tested as vaccines. Efforts to purify the toxin are in progress, and sedimentation studies of the partially purified toxin by means of the optical ultracentrifuge have so far revealed two major components which we expect to separate by physical-chemical methods.

The bacterial protoplasm alone gave little protection and was nontoxic. That it contains antigenic substances, however, is shown by relatively high precipitation titers with immune sera. Thus, a considerable advantage was gained by the preliminary separation of the more active cell walls from the protoplasm, which, although chemically complex, is of little immunologic significance.

Significance to Microbiological Research:

The so-called endotoxins which numerous investigators have extracted or separated from Salmonella and other gram-negative bacteria have contained the antigenic activities associated with precipitation,antibody stimulation, toxicity, and, somewhat questionably, protection. It has been concluded, therefore, that the isolated endotoxin complex is itself the somatic antigen complex of the bacterium. It appears obvious that methods involving extraction of intact cells with trichloracetic acid (Boivin) or uncontrolled mechanical disintegration of the cells will result in preparations which are a mixture of different antigens, the separation of which would be very difficult. Boivin-type antigens have not been found useful as vaccines, because they are rich in toxin, yet poor in protective antigen.

These studies attempt to re-examine certain accepted concepts of antigens in the hope that new information will be obtained. It is believed that new methods for isolating effective and reasonably pure antigen may result, making it possible to increase the amount of immunizing material in vaccines while decreasing undesirable side reactions. Satisfactory immunization against typhoid fever and Salmonella infections in man and animals would be contemplated.

Proposed Course of Project:

To continue, along lines outlined above, in efforts to produce a detoxified vaccine and to purify and characterize the major antigens concerned in immunity to salmonellosis; and to apply the methods so far developed to study of the typhoid bacillus. The scope of the project will be extended to include other infectious agents which there is reason to believe may be susceptible to the same type of analysis. In particular, work with Mycobacterium tuberculosis is in the planning stage.

(This project is a continuation of earlier projects on separation of antigens in the Biophysics Unit and on experimental salmonellosis in the Enteric Disease Unit.)

R.P.C. - 2
December 1955

Analysis of NIH Program Activities

SERIAL NO. NMI-69

Budget Data Sheet

10.

11.

12. BUDGET ACTIVITY:

RESEARCH

13. None

14. None

Analysis of NIH Program Activities SERIAL NO. NMI - 69

Honors, Awards, and Publications Sheet

15.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

1. Hoyer, Bill H., and Courdurier, Jean: Detection of plague antigens in tissues of persons dead from plague. Bull. Soc. Path. exot. 47: 758-759. Nov.-Dec. 1954.

17. None

R.P.C. - 1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

1. National Microbiological Institute
INSTITUTE

2. Rocky Mountain Laboratory
LABORATORY OR BRANCH

3. None

4. Hamilton, Montana
LOCATION

5. NMI - 76
SERIAL NO.

6. Study of the Amino Acid Metabolism of Normal and Virus-Infected Tissue-Culture Cells
PROJECT TITLE

7. Dr. Bill H. Hoyer
PRINCIPAL INVESTIGATOR

8. Daniel B. Ritter
OTHER INVESTIGATOR

9. PROJECT DESCRIPTION

Objectives:

To determine the combined host-parasite induced metabolic changes which occur when viruses infect susceptible tissue-culture cells.

Methods Employed:

Radioactive glucose and fructose randomly labelled with C^{14} are fed to normal and virus-infected cells. The virus-infected cells are fed C^{14} at various intervals. In order to promote incorporation of the radioactivity, normal and infected cells are overlayed with glucose and serum-free Hanks' solution. The incorporated C^{14} trichloracetic acid (TCA) precipitable C^{14} compounds are hydrolyzed, and the hydrolysate is subjected to two dimensional paper chromatography. Radioautographs are made from the chromatograms, and the radioactivity of the isolated compounds is determined by counting their activities directly from the chromatogram.

Major Findings:

Normal monkey kidney and HeLa cells incorporate C^{14} from randomly labelled glucose-fructose into TCA precipitable compounds which contain C^{14} aspartic acid, C^{14} glutamic acid, C^{14} alanine, and C^{14} valine. The ratio of counts from valine/counts from glutamic acid is about 0.25; from alanine/glutamic acid about 0.30; from valine/alanine about 0.9. During the final stages of infection of monkey cells the C^{14} continues to be incorporated into TCA precipitable valine-containing compounds but is not incorporated into TCA precipitable glutamic acid or alanine compounds at the normal levels. Therefore, in cells infected with poliomyelitis and vaccinia viruses the ratio of counts from valine/counts from glutamic acid is 8-20; from alanine/glutamic acid about 0; from valine/alanine about 20 to infinity.

Significance to Microbiological Research:

In order to incorporate C^{14} from glucose or fructose into TCA precipitable compounds which contain amino acids, a minimum of ten separate enzymatic conversions must take place. This series remains nearly intact during the final stages of polio and vaccinia infections of monkey kidney cells insofar as the metabolic steps leading to valine formation are concerned. The chain of steps is unlinked somewhere in the conversion of these sugars to alanine and glutamic acid. Thus, in virus-infected cells it is possible to demonstrate an effect peculiar to the host-virus relationship of the polio or vaccinia viruses. These findings, thus, contribute to the partial understanding of some of the intimate host-parasite relationships in these systems.

Proposed Course of Project:

The project concerned with the metabolism of animal virus-tissue culture cell systems is completed. Attempts were made to extract soluble proteins from tissue-culture cells which would be suitable for paper electrophoretic studies of intact cell proteins during infection, but experimental difficulties were encountered. In order to check our results with animal viruses in another system, it is proposed that studies similar to the above be done with bacteriophage systems.

It is also planned to use similar techniques in the study of geriatrics by the use of young and aged bacterial cells. Using radioactive tracers, the metabolic reactions taking place in cells from the logarithmic growth phase and aged cells will be compared. The use of deficient mutants may be very valuable in some phases of such a study. Amino acids, sugars, and simple compounds will be identified from hydrolysates by means of paper chromatography, and studies of soluble protein components will be made by paper electrophoresis. Studies will be attempted on "resting" cells so growth and reproduction will not be considered. An understanding of the aging processes is increasingly important and will become more so as our public health and medical practices improve. Since the bacterial cell provides a very suitable model for the short-term study of aging, it is proposed that such information be obtained from this source.

R.P.C. - 2
December 1955

Analysis of NIH Program Activities

SERIAL NO. NMI -70

Budget Data Sheet

10.

11.

12. BUDGET ACTIVITY:

RESEARCH

13. None

14. None

R.P.C. - 3
December 1955

Analysis of NIH Program Activities SERIAL NO. NMI -70

Honors, Awards, and Publications Sheet

15.

16. None

17. None

R.P.C. - 1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

1. National Microbiological Institute
INSTITUTE

2. Rocky Mountain Laboratory
LABORATORY OR BRANCH

3. None

4. Hamilton, Montana
LOCATION

5. NMI
SERIAL NO.

6. The Relationship between Viruses and Tumors
PROJECT TITLE

7. Dr. Richard A. Ormsbee
PRINCIPAL INVESTIGATOR

8. Dr. Lawrence Kilham, Dr. William J. Hadlow, Robert K. Gerloff, and Dr. Willy Burgdorfer
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives:

To explore the possible etiologic relationships of viruses to tumors, to develop malignant from benign growths, to determine the pathogenesis of virus-induced tumors, to determine the relations between viruses and host cells in terms of metabolic activity and physical change, to discover oncolytic agents which are not deleterious to the health of tumor-bearing animals, and to elucidate the relation of latent viruses to cancer.

Methods Employed:

Tissue cultures will be employed in many of these studies and so-called "orphan viruses" of human origin will be tested for activity against certain tumors known to be induced by viruses. Transplantable tumors in rats will be employed as test material to determine the oncolytic effects of certain viruses. Arthropod transmission of certain virus-induced tumors will also be studied to elucidate virus-tumor relations. The relation of serum antibodies, tissue immunity, age, pregnancy, and other factors to the persistence of virus in tumors, to tumor growth and regression, and ability to metastasize will be investigated. A wide-angled approach, employing many diverse aspects of microbiological methods, is visualized.

Major Findings:

Encephalomyocarditis virus causes regression of gastric carcinoma 344, osteogenic, in inbred rats of M520 strain. Fibroma virus may be transmitted by mosquitoes with ease between eastern cottontail rabbits but not between domestic rabbits or western cottontail rabbits. Fibroma virus when grown in squirrels produces a disease characterized by extensive metastases. Very much larger tumors are produced by this virus in western than in eastern cottontail rabbits. Fibroma virus has been adapted to intravenous passage and produces extensive tumor spread.

Significance to Microbiological Research:

The significance of the above specific results demonstrates that another virus is capable of causing regression of animal tumors. The possibility that certain virus skin tumors may have evolved in association with insect vectors is demonstrated by transmission of virus-induced tumors by mosquitoes. The variable reaction of fibromas in various host species and in the same host injected by various methods suggests that the virus is responsive to selective processes within the host animal. In general, it is considered that the conclusion that tumors may be induced by viruses is an evident one, and extension of knowledge of virus-tumor relations should be made as rapidly as possible.

Proposed Course of Project:

It is proposed to continue to attempt to determine factors which cause benign viral tumors to become malignant, to determine the relations which exist between insect vectors and the viruses responsible for tumors, to discover other transmissible tumors of animals, to study the growth of various tumors and tumor-inciting viruses in tissue cultures, to attempt to demonstrate viral etiology of tumors not presently known to be virus-induced, to determine the metabolic relations of cells infected with such viruses in comparison to normal cells, and to search for oncolytic agents employing tissue-culture methods and intact animals.

R.P.C. - 2
December 1955

Analysis of NIH Program Activities

SERIAL NO. NMI

Budget Data Sheet

10.

11.

12. BUDGET ACTIVITY:

RESEARCH

13. None

14. None

R.P.C. - 3
December 1955

Analysis of NIH Program Activities

SERIAL NO. NMI

Honors, Awards, and Publications Sheet

15.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

1. Kilham, Lawrence, Mason, P., and Davies, J. N. P.: Pathogensis of fatal encephalomyocarditis (EMC) virus infection in albino rats. Proc. Soc. Exp. Biol. & Med. 90(2): 383-387. Nov. 1955.

In Press

1. Kilham, L., Mason, P., and Davies, J. N. P.: Host-virus relations in encephalomyocarditis (EMC) virus infections. I. Infections of wild rats. Amer. J. Trop. Med. & Hyg.
2. Kilham, L., Mason, P., and Davies, J. N. P.: Host-virus relations in encephalomyocarditis (EMC) virus infections. II. Myocarditis in mongooses. Amer. J. Trop. Med. & Hyg.
3. Kilham, Lawrence, Habermann, R. T., and Herman, C. M.: Jaundice and bilirubinemia as manifestations of canine distemper in raccoons and ferrets. Amer. J. Vet. Res.

17. None

R.P.C. - 1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

1. National Microbiological Institute
INSTITUTE

2. Rocky Mountain Laboratory
LABORATORY OR BRANCH

3. None

4. Hamilton, Montana
LOCATION

5. NMI
SERIAL NO.

6. FCDA Project: Investigation of the Efficacy of Multivalent Antigens
PROJECT TITLE

7. Dr. Carl L. Larson
PRINCIPAL INVESTIGATOR

8. Dr. Cora R. Owen
OTHER INVESTIGATOR

9. PROJECT DESCRIPTION

Objectives:

To study the effects upon the subsequent immunity of combining the antigens of various infectious or toxigenic agents into a single immunizing dose.

Methods Employed:

Mice were injected with the antigen of Pasteurella tularensis, P. pestis, Histoplasma capsulatum, Blastomyces dermatiditis, and Clostridium botulinum, or exposed to influenza A virus in the cloud chamber, alone or in combinations, and later challenged with the agents. The death rates were observed and compared.

Major Findings:

No differences were found in the degree of immunity or in the rate of its development to P. tularensis, H. capsulatum, B. dermatiditis, Cl. botulinum, or influenza A virus when either the monovalent or the multivalent antigens were employed. However, when animals which had been vaccinated with multivalent antigens containing the antigens of H. capsulatum, particularly, or B. dermatiditis, were challenged with P. pestis, a higher degree and more rapid development of immunity, as compared to animals vaccinated with only P. pestis antigen, was a constant finding.. Incorporation of this last antigen into the fungal antigens had no effect. Preliminary attempts to establish the part of the fungal cell in which this heterologous antigen was situated were negative.

Significance to Microbiological Research:

Incorporation into a single immunizing dose of antigens of the most diverse types, provenances, and degrees of purity does not affect the development in mice of immunity to the causative agents. The exception to this was an enhancement of immunity to *P. pestis* from the crude antigens of *H. capsulatum* or *B. dermatiditis*. This represents two further examples of cross immunity or of common antigens between fungi and bacteria.

Proposed Course of the Project:

This project is now terminated.

R.P.C. - 2
December 1955

Analysis of NIH Program Activities

Budget Data Sheet

SERIAL NO. NMI 73

10.

11.

12. BUDGET ACTIVITY:

RESEARCH

13. Supported by F.C.D.A. funds.

14. None

R.P.C. - 3
December 1955

Analysis of NIH Program Activities SERIAL NO. NMI

Honors, Awards, and Publications Sheet

15.

16. None

17. None

R.P.C. - 1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

1. National Microbiological Institute
INSTITUTE

2. Rocky Mountain Laboratory
LABORATORY OR BRANCH

3. None

4. Hamilton, Montana
LOCATION

5. NMI 14
SERIAL NO.

6. Morphologic Changes in Virus-Infected Monkey Kidney and HeLa Cells
PROJECT TITLE

7. Dr. Bill H. Hoyer
PRINCIPAL INVESTIGATOR

8. Nick J. Kramis and Daniel B. Ritter
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives:

To determine the sequence and type of morphologic changes which occur in virus-infected monkey kidney and HeLa cells in mono-layer tissue cultures in order to better understand the nature and kinetics of virus infections.

Methods Employed:

Time-lapse phase cinemicrography has been used to photograph the sequence of changes resulting when HeLa and monkey kidney cells are infected with poliomyelitis, vaccinia, and APC viruses. The resulting pictures constitute a 16 mm. motion picture film which gives a condensed and clear picture of the type of changes which occur when these tissue-culture cells are virus-infected, and enables the accurate determination of the time required for each change.

Major Findings:

Normal cells placed in unbalanced salt solutions which promote cell starvation or rapid rounding do not go through the same changes which are induced by the viruses studied. Also, normal cells placed under the same conditions used for infected cells remain unaffected.

Monkey kidney cells infected by polio and vaccinia viruses go through the same type of changes, namely: (1) A preliminary rounding or thickening of the cell which may be accompanied by some cytoplasmic stringing and (2) violent bubbling which resembles violent extrusion of many pseudopods. Then (3) the extruded portions are drawn into the finally rounded cell which, (4) undergoes the final degenerative changes which result in ultimate cell breakdown.

HeLa cells infected by polio and vaccinia viruses show (1) rounding followed by (2) rapid bubbling which gives the rounded cell a warty appearance and (3) cessation of bubbling in which the extruded portions are not withdrawn but which (4) gradually break off from the rounded body and fill the supernatant fluid with many small globules. Gradual degenerative changes then follow.

HeLa and monkey kidney cells infected with APC virus show (1) initial rounding followed by (2) a clumping of the cells and (3) a fusion of the individual cells into undifferentiated clumps. The consistent feature of bubbling found in the polio and vaccinia viruses is rarely found in APC infected cultures.

Significance to Microbiological Research:

These studies give a clearer idea of the mechanisms involved in cell destruction by the viruses studied. Too, it is possible to detect well defined stages of infection (i.e., bubbling) which will be valuable in studies of the kinetics of virus infection. Also, the resulting motion pictures are valuable visual education aids.

Proposed Course of Project:

Preparation of a motion picture by N. J. Kramis for general distribution is under way. Other viruses and cell systems will be studied by this technic, and the kinetics of infections involving various virus-cell proportions is contemplated.

R.P.C. - 2
December 1955

Analysis of NIH Program Activities

Budget Data Sheet

SERIAL NO. NMI 74

10.

11.

12. BUDGET ACTIVITY:

RESEARCH

13. None

14. None

Analysis of NIH Program Activities SERIAL NO. NMI 74

Honors, Awards, and Publications Sheet

15.

16. None

17. None

R.P.C. - 1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

1. National Microbiological Institute
INSTITUTE

2. Rocky Mountain Laboratory
LABORATORY OR BRANCH

3. None

4. Hamilton, Montana
LOCATION

5. NMI
SERIAL NO.

6. The Relation of Antibodies, Immunity, and Hypersensitivity
PROJECT TITLE

7. Dr. Samuel B. Salvin
PRINCIPAL INVESTIGATOR

8. Dr. Edgar Ribi and Dr. Carl L. Larson
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives:

The objectives of this project are concerned with the role of antibodies in sensitivity, the role of delayed and immediate types of hypersensitivity in immunity, the nature of antibody in certain types of immunity, and the site of origin and the nature of antigens obtained from allergenic pollens.

Methods Employed:

Corynebacterium diphthereae and *Diplococcus pneumoniae* and products obtained from these organisms are utilized to produce allergic reactions in animals. Tissue-culture methods and classical immunochemical techniques are employed in the analysis of the entity induced. In order to study the site of origin and the nature of antigens in allergenic pollens, chemical, physical, and immunologic methods will be employed as has been done with various bacterial and fungal agents in this laboratory.

Major Findings:

These projects are just being initiated or planned and no results are available.

Significance to Microbiological Research:

The problems of allergy are complex, yet research is imperative as these represent some of the most common and aggravating of human ailments. In order to understand allergy and hypersensitivity, the

7

Significance to Microbiological Research (Continued)

type of circulating antibody associated with allergic diseases, the mechanism of the sensitizing process, and the site of antibody-antigen reaction must be clarified.

The antigens ordinarily employed in skin testing and in desensitization of allergic patients are at least as crude as those used for immunization against bacterial and viral infections. Studies in this laboratory have shown that nontoxic immunogenic fractions can be obtained from cell walls of certain fungi and bacteria by physical methods. Such antigenic analyses could result in separation of antigens which are toxic and useful for diagnostic purposes and antigens which are nontoxic and useful for desensitization and immunization of allergic individuals.

Proposed Course of Project:

These projects are either just starting or are being initiated. It is planned to study vigorously these aspects of antibody and antigen in relation to allergy and to employ the results to guide further studies in the field.

Budget Data Sheet

10.

11.

12. BUDGET ACTIVITY:

RESEARCH

13. None

14. None

R.P.C. - 3
December 1955

Analysis of NIH Program Activities SERIAL NO. NMI 75

Honors, Awards, and Publications Sheet

15.

16. None

17. None

R.P.C. - 1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

1. National Microbiological Institute
INSTITUTE

2. Rocky Mountain Laboratory
LABORATORY OR BRANCH

3. None

4. Hamilton, Montana
LOCATION

5. NMI 76
SERIAL NO.

6. Spread of Viruses in Human Populations, Particularly Nonimmune
PROJECT TITLE

7. Dr. Carl M. Eklund
PRINCIPAL INVESTIGATOR

8. Dr. Carl L. Larson, Dr. E. John Bell, and Robert K. Gerloff
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives:

To study disease outbreaks in sparsely populated localities and in very isolated areas. The location of this Laboratory permits access to such areas in the Northwest and in Alaska. Here the opportunities for studying the spread of infectious agents are far superior to those found in densely settled areas where the incidence of immunity is high and the tracing of an infection chain very difficult.

The unfortunate occurence in Idaho during the early stage of the poliomyelitis immunization program permitted a study of the effects produced by this accident. An attempt was made to determine the amount of virus given; the incidence of clinical disease produced, and its clinical features; whether carriers were produced and if so, how many; how extensive an epidemic was produced, and its duration.

Methods Employed:

1. Devise procedures for the detection of virus in vaccines.
2. By collection of stool and blood specimens from patients and family contacts, determine number of cases produced, and get an estimate of carriers produced.
3. By serological studies on vaccinated children, get an estimate of number of infections produced and immunity resulting.

Major Findings:

Twenty cases of poliomyelitis occurred in vaccinated children soon after administration of the vaccine, most within 5-14 days. These cases were characterized by an unusual incidence of upper-extremity paralysis. Up to June 15, only Type I virus was isolated from Idaho patients and their contacts and nearly every case occurred in a vaccinated child or a close associate of a vaccinated child.

Using the data obtained from the control children in the 1954 field trials to estimate the number of children with antibodies prior to vaccination, it appears that of children with no previous antibodies, 42% acquired Type I, 50% Type II and 30% Type III after vaccination. There are large sampling errors involved, but a conservative estimate appears to be that 50% of children with no previous immunity acquired immunity after administration of the vaccine used.

It has been learned that the monkey test is more valuable in testing vaccines than was formerly supposed.

Proposed Course of Project:

The present project involves further work on the relation between a monkey paralytic dose of virus and a tissue-culture dose of virus. Some sera and stool specimens remain to be examined. Similar studies will be carried out on sera collected from vaccinated and non-vaccinated children in selected towns in Montana. The type of poliomyelitis infection which occurs in Idaho should be followed for some time to determine whether or not a seeding of an area with Type I virus may influence the type of infection to be expected for several years in the future. The results of the study of the Pribilof and Idaho outbreaks have been of such interest that a study project, which appears to be only opportunistic, is amply justified.

Note: In connection with studies of poliomyelitis vaccine, Dr. Robert N. Philip was assigned by the Division of Biologics Standards to collect blood specimens from vaccinated and nonvaccinated children in selected towns in Montana in an attempt to determine whether there was live virus in the vaccine used. These specimens will be processed and tested at the Rocky Mountain Laboratory.

Analysis of NIH Program Activities

Budget Data Sheet

10.

11.

12. BUDGET ACTIVITY:

RESEARCH

13. None

14. None

Honors, Awards, and Publications Sheet

15.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

1. Bodian, David, Francis, Thomas Jr., Salk, Jonas E., Shope, Richard E., Larson, Carl L., Smadel, Joseph E., and Shannon, James A.: Interim report PHS technical committee on poliomyelitis vaccine. J.A.M.A. 159(15): 1444-1447. Dec. 10, 1955.

In Press

1. Eklund, Carl M., and Larson, Carl L.: Outbreak of Type III poliomyelitis on St. Paul Island, Alaska. Amer. J. Hyg.

17. None

R.P.C. - 1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

1. National Microbiological Institute
INSTITUTE

2. Rocky Mountain Laboratory
LABORATORY OR BRANCH

3. None

4. Hamilton, Montana
LOCATION

5. NMI -
SERIAL NO.

6. Studies on Poliomyelitis Vaccine with Emphasis on the Number of Virus Particles Contained in Vaccine Suspensions and on a Modified Monkey Test for the Detection of Residual Virus in Vaccines
PROJECT TITLE

7. Dr. Carl M. Eklund
PRINCIPAL INVESTIGATOR

8. Dr. Edgar Ribi, Dr. Bill H. Hoyer, and Daniel Ritter
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives:

To determine the numbers of physical particles from various virus preparations in reference to their tissue-culture titers; to detect any residual live virus in formalinized poliovirus vaccines by using specially treated monkeys of various species and tissue-culture cells obtained from these species.

Methods Employed:

Various purification methods will be attempted in order to remove nonvirus material from the vaccine preparations to be studied. The numbers of physical particles present in the preparation will be determined by the spray-droplet technic of Backus and Williams.

Monkeys are treated with cortisone at varying intervals before and after inoculation and, if apparatus becomes available, with deep x-ray. Routes of inoculation are explored--intralumbar at multiple or single sites, intratonsillar, brown fat pads, and into inguinal nodes. Attempts are made to demonstrate virus in serial blood specimens, tonsils, lymph nodes, and brown fat.

The project is just initiated, but preliminary survey has indicated that much denatured and clumped material is present in electron-microscope preparations of ordinary commercial vaccines.

Cortizonized monkeys appear superior to tissue culture in detecting virus in vaccine. In untreated virus suspensions, only green monkeys seem to be the equal of, or even superior to, tissue culture in detection of virus.

Significance to Microbiological Research:

The progress made in detecting small amounts of live virus in the presence of large amounts of formalin in activated poliovirus and in counting virus particles offers hope that the nature of vaccines prepared with minimal amounts of formalin can be determined and thus lead to the production of safer and more efficatious virus vaccines.

Proposed Course of Project:

Direct counts, by means of electron microscopy, of poliovirus particles, and others, with special reference to vaccines will be continued. Further tests will be made with cortizonized and green monkeys.

R.P.C. - 2
December 1955

Analysis of NIH Program Activities

Budget Data Sheet

SERIAL NO. NMI

10.

11.

12. ~~BUDGET ACTIVITY:~~

RESEARCH

13. None

14. None

R.P.C. - 3
December 1955

Analysis of NIH Program Activities

SERIAL NO. NMI 77

Honors, Awards, and Publications Sheet

15.

16. None

17. None

R.P.C. - 1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

1. National Microbiological Institute
INSTITUTE

2. Rocky Mountain Laboratory
LABORATORY OR BRANCH

3. None

4. Hamilton, Montana
LOCATION

5. NMI 78
SERIAL NO.

6. Community Health Project
PROJECT TITLE

7. Dr. J. Frederick Bell
PRINCIPAL INVESTIGATOR

8. Dr. E. John Bell, Dr. Kelsey C. Milner, Dr. Carl L. Larson, Dr. Richard A. Ormsbee, R. K. Gerloff, Dr. Carl M. Eklund, Dr. David B. Lackman, Dr. W. J. Hadlow, Dr. Lauri Luoto, and Dr. W. H. Gaub
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives:

The objectives of this program are to discover the major causes of febrile illnesses in a rural community, to determine the type of illness associated with specific agents, to develop diagnostic methods, to elaborate the epidemiology of these illnesses and to discover methods for the specific control and prevention of such diseases. Many studies are being directed toward certain of these objectives in urban centers, institutions of various types, and in restricted populations in heavily populated areas. These do not offer opportunities which are afforded by studies being carried out in less heavily populated areas. Furthermore, the findings in heavily populated areas may not be applicable to an area such as this one. Hamilton, Montana, serves as a fairly representative western rural community, and findings here may well be applied generally to such communities. Attempts also will be made to isolate and propagate the causative agent of infectious hepatitis from this or other communities.

A major portion of the facilities originally developed for this project were shifted into a study of poliomyelitis as it occurred in Idaho during 1955 following use of Cutter vaccine and to a study of the effects of vaccine upon excretion of poliovirus and development of antibodies.

Methods Employed:

The use of epidemiologic and clinical approaches to the problems of poliomyelitis depends upon the nature of the problem presented in Idaho in relation to the Cutter incident and to the testing of a lot of vaccine in Montana where it was desired to determine the relation of antibody production to the presence or absence of virus in children.

Serological approaches include the use of tissue-culture neutralization tests for poliovirus and related agents, complement-fixation tests for APC viruses (ARD), and neutralization tests for Coxsackie viruses.

Etiological studies include the use of a wide variety of cells grown in tissue culture, embryonated eggs, suckling mice, monkeys, and selected bacteriological media.

In addition to sampling the population by examination of individual stool and sputum specimens, samplings have been carried out by means of community serological tests and by "swabbing" the sewers.

The study therefore employs clinical, epidemiological, serological, and microbiological methods that are pertinent to the problem.

Major Findings:

1. During the summer, fall, and early winter an epidemiological study of the effects of the use of contaminated vaccine was carried out in Idaho. The incidence of cases was studied and the relation of secondary to primary cases investigated.

2. During 1955 a total of 3,678 specimens was submitted for examination. These specimens included 1253 associated with the Idaho outbreak and 603 for the study of the effects of poliomyelitis vaccine in Montana children. From this material there have been 643 isolations of poliovirus and 133 isolations of viral agents other than poliovirus. In addition, 105 *Salmonella* have been obtained (including 3 of *S. typhi*), 10 paracolon bacilli, and 1 *Shigella flexneri*.

3. Coxsackie viruses A 1, 2, 3, 4, 5, 6, 7, 8, 10, and B 2 and 4 have been isolated from the area. Strain A 8 appears to be the epidemic strain in Hamilton, Mont. There is a marked correlation between illness and isolation of these agents and between age and development of antibodies to Coxsackie viruses. The marked correlation between diarrheal disease and the presence of these agents suggests a causal effect.

4. There is an increasing incidence of antibodies against distemper virus in relation to age among residents of Hamilton.

5. Approximately 50% of 202 school children had antibodies against APC viruses (ARD).

6. Among preschool children, the incidence of antibodies against Type I, II, or III poliovirus was 23%, 23%, and 8%, respectively, while among high school students the incidence was 52%, 41%, and 36% for the three types of poliovirus.

7. A study of the sewers in Hamilton shows that the isolation of poliovirus is greatest during the late fall and winter months and the least during June, July, and August. APC viruses were isolated in July and August, whereas other agents were present throughout the year.

8. Of several tissue-culture systems tried in attempts to isolate the agent of infectious hepatitis from serum of human cases, epithelial cells from human lymphatic tissue have given the most promise with suggestions of cytopathologic changes.

Significance to Microbiological Research:

The studies in Idaho are concerned with a situation which is unparalleled for understanding of the epidemiology of poliomyelitis. It is probable that another such situation will never develop. The studies of the effect of vaccine upon virus excretion and upon production of antibodies should demonstrate whether or not the production of antibodies by individuals vaccinated with formaldehyde-inactivated material is dependent upon the presence of live virus.

A study of febrile illnesses in an isolated rural population to determine the cause of these diseases and their impact upon the community is highly desirable, since a great segment of our population lives in such communities and the factors that influence the incidence and spread of disease in rural towns may not be similar or identical to those in urban areas or in institutions. Since it is possible to study epidemiologic facts in a town such as Hamilton without undue interference caused by the pressure of heavy populations, the correlation of clinical, epidemiological, etiological, and serological data can be made more efficiently. The varying incidence of certain viruses in sewer swabs in relation to the presence of disease suggests that spread of infection occurs at a considerable time before illness becomes manifest.

Proposed Course of Project

During the ensuing year the material accumulated from investigations in Idaho and on the vaccine problem in Montana will be tested to completion and this phase of the project terminated.

The community study in Hamilton will be continued and strengthened by the return of facilities employed in the poliomyelitis vaccine investigations. Due to interruptions beyond our control, we were unable to assemble the population core which was to be used for intensive study, nor were we able to fully develop methods to handle the problem. It is hoped that this will be accomplished in the near future so that statistical data can be obtained. Specific attention will be given to methods for isolation of the etiological agent of infective hepatitis, and attention will be directed toward the immunology of certain of the viruses isolated to date.

R.P.C. - 2 Analysis of NIH Program Activities SERIAL NO. NMI
December 1955

Budget Data Sheet

10.

11.

12. BUDGET ACTIVITY:

RESEARCH

13. None

14. None

R.P.C. - 3
December 1955

Analysis of NIH Program Activities

SERIAL NO. NMI

Honors, Awards, and Publications Sheet

15.

16. None

17. None

NATIONAL INSTITUTE OF DENTAL RESEARCH

SUMMARY OF RESEARCH ACTIVITY

January - December 1955

The objectives of the programs administered by the National Institute of Dental Research are to increase knowledge of diseases and impairments of the oral cavity and associated structures and to improve the oral health and, thus, the general health of the Nation. The program of the Institute is divided into three broad categories: (1) a research program conducted directly by the staff of the Institute, including projects in both laboratory and clinical investigations, (2) a program designed to support scientific research projects being conducted in non-Federal institutions, and (3) a program to encourage and increase the Nation's potential of scientists qualified to carry out dental research.

A review of recent accomplishments under our program of dental research reveals evidence of a very significant and extremely gratifying trend. It is now apparent that dental research is undergoing a definite shift in emphasis from concern with the development of practical procedures for alleviating dental disease and perfecting dental restorations to a search for knowledge of a more fundamental character. The common denominator of all biological research is the employment of the scientific method for determining the physiological responses of the living cell to various nutritional, functional and environmental changes. Under this concept, the only difference between the work of the dental researcher and other researchers in the biological field would be in the site of the tissues and organs selected for study.

LABORATORY RESEARCH

ORAL AND BIOLOGICAL CHEMISTRY:

Research in this area includes investigations of nutritional and biochemical factors in the etiology of experimental caries; biochemistry of dentin, enamel and periodontal tissues; fluorine metabolism and physiological effects; and saliva chemistry.

1.. Nutritional and Biochemical Factors: Dietary studies to date emphasize the possibility that heat processing of protein foods (skim milk powders and cereal foods especially) is involved in the production of dental caries in the white rat. Basic reactions identified with the browning reaction of heat processing may be involved, as well as the loss of essential amino acid lysine. Laboratory research, therefore, applies to basic reactions of reducing sugars and lysine.

Effects of caries inhibiting agents may give clues to the etiology of caries and may provide practical means of caries control. Agents studied include various fluorides, iodoacetic acid, sodium lauryl sarcosinate, excess of complex salt mixture, and extra fat in the diet. Caries potentiating agents being studied are ethylene diamine tetraacetic acid, and dehydroacetic acid. The latter compound, because of its toxic effects, may give leads to systemic factors related to the development of dental caries.

2. Biochemical Studies of Oral Tissues: The work is being organized to evaluate chemical and enzymatic changes in oral soft tissues in relation to their pathology. This fundamental problem of diseases of the oral soft tissues is one about which little, if any, information is available. Satisfactory methods are being developed for analysis of glycogen tyrosine, hydroxyproline, hexosamine total, and amino nitrogen. Other studies concern methods for inducing periodontal disease and inflammation in oral soft tissues of the white rat.

Dental caries probably is related to the chemistry of dentin and enamel. The proteins and amino acids of the organic matrix of these tissues are being studied by ion exchange techniques; analytical procedures perfected to provide separation of diastereoisomers; pH gradient elution investigated for application to the Moore and Stein method of amino acid separation.

3. Fluoride Metabolism Studies: These researches on fluorine metabolism and its physiological effects have the primary purpose of obtaining basic knowledge to aid in evaluating the relation of fluoridated water to public health. These studies include: (a) Experiments with laboratory animals to evaluate the skeletal deposition of fluorine as influenced by age and inanition, and the distribution of fluorine in different bones and within the same bone. (b) Experiments using radioactive calcium to determine the effect of fluorine on calcium metabolism in skeletal tissues. Translocation of skeletal fluorine, after withdrawal of fluorine, is a phase of fluorine metabolism under consideration.

4. Saliva: The chemistry of saliva has a high potential as a factor both in caries etiology and periodontal disease. This secretion is being studied in the following areas: amino acids; non-volatile, non-nitrogeneous organic acids; and electrolytes, particularly sodium and potassium.

ORAL MICROBIOLOGY:

The research projects in this area have two major long-range objectives:

1. To build a fund of basic scientific information about the oral microflora and individual types of bacteria associated with dental disease. This project includes the enumeration of different types of oral microorganisms, their isolation in pure culture, their taxonomic characterization, their enzymes, their nutritional requirements, and the biochemical reactions they produce.

2. To determine and define more adequately the etiological relationships which may exist between oral microorganisms and oral and systemic diseases. Infections with oral organisms generally are not dramatic. The demonstration of their cause-and-effect relationships requires new techniques and basic developments. It is possible that different types of microorganisms might lead to the development of disease, providing other factors in the environment are predisposing. Therefore, etiological studies must include the interrelationships of many different factors in order to lay an adequate foundation for solution of this problem.

HISTOLOGY AND PATHOLOGY:

The studies in this area are divided into the following categories: morphological, histochemical, and histopathological.

1. Morphological Studies: This program has as its objectives the development of fundamental information concerning the characteristics and properties of normal and pathologically affected oral hard and soft tissues. A major project has been the application of physical methods (primarily electron microscopy) to the study of dentin and enamel. Emphasis has been on technical methods and the derivation of information regarding the sub-microscopic structure of oral soft tissues, enamel, dentin. An important area of present investigation pertains to the form, size, orientation and development of the sub-microscopic crystalline component of the hard tissues.

2. Histochemical Studies: Fundamental studies are being conducted to characterize histochemically protein components and enzyme activities (primarily peptidase and esterase) in oral and other soft tissues. The histochemical properties of connective tissues in general are also under investigation. The latter studies relate to periodontal diseases and deteriorations which occur with advancing age. Other studies pertain to the mineral metabolism of bone, using optical microscopy, radiotracer techniques, chemical analyses and physical measurements.

3. Histopathological Studies: Basic pathological phenomena as reflected by structural changes in tissue are studies by cytologic and histologic methods, interest centering upon the sequence of events occurring in periodontal diseases. The oral mucous membrane is also being investigated, emphasis being placed upon determining: (1) the limits of structural variation in clinically normal tissues (for use in diagnostic practice), (2) the oral manifestations of systemic disease, and (3) the effects of therapy with various drugs and chemical agents.

EPIDEMIOLOGY AND BIOMETRY:

These research activities are devoted to two broad fields of investigation, which may be subdivided as follows:

1. The fluoride-dental caries relationship is being studied to

test two hypotheses: One is, that dental caries can be controlled by the use of NaF to adjust the fluoride level of a community water supply. This hypothesis is being evaluated by a study in which the fluoride level of the community water supply of a study city has been adjusted to 1 ppm by the controlled addition of NaF.

The second hypothesis, that the fluoride ion is effective if supplied by a compound other than NaF, is being tested by a study in which the water supply of the study area has been fluoridated with sodium silicofluoride to a level of 1 ppm F. This study has been in progress four years.

2. In addition, the development of a satisfactory method for quantitatively estimating the severity and extent of periodontal disease has been a major problem for this section. A new system designed to measure inflammatory types of periodontal disease has been developed and is in use. Data are being gathered in the dental caries-fluoride studies as well as in studies specifically designed to measure periodontal disease in older age groups.

CLINICAL INVESTIGATIONS

The Dental Institute's activities in this area were begun in 1954. During this period, a large portion of the program was centered on cooperative studies with the previously mentioned research programs. In addition to these cooperative research activities, the following clinical studies were in operation.

1. Studies in growth and development of the skeletal tissues of the face and head. These are longitudinal studies of abnormal patterns of growth of the head in a large series of newborn children. In progress since 1949, this project is being carried on jointly with the Division of Services for Crippled Children and the Cleft Palate Center of the University of Illinois. The purpose of this research is to derive basic information pertaining to the post-natal growth and development of the head during the early years of prolific growth. It is anticipated that information derived from these studies will be supportive to the design of therapy for children so afflicted and will increase the ultimate understanding of these anomalies.

2. Studies of general systemic effects of fluorides: Controlled metabolic and fluoride balance studies on patients in the Clinical Center. Fluoride exchange studies are being conducted on normal controls and patients with either hypertensive cardiovascular disease or primary renal disease. An epidemiological human autopsy study was initiated to obtain detailed information on the histology and chemical composition of bone and soft tissues of persons who had lived in high-fluoride areas for long periods. Histological and chemical studies are being made of tissues obtained.

3. Emphasis is being given also to investigation of genetic factors in the development of oral structures, particularly malformation of dental tissues. A study just initiated concerns the genetic significance of certain associated dental, auditory, kidney and other defects in a group of 4,000-5,000 individuals, all related to each other, on whom exhaustive pedigree histories can be obtained.

EXTRAMURAL PROGRAM

RESEARCH GRANTS:

There seems little question but that the dental research potential is today far in excess of available funds to support such research. In the absence of interest on the part of the voluntary foundations to provide for this support, it becomes a duty of the Institute to take steps to alleviate the situation. The problem differs from that of other categorical Institutes in that the dental research program does not have the advocacy of any large effective national organization other than its own professional group.

At the June 1954 meeting of the National Advisory Dental Research Council it was apparent that dental research was not only lagging far behind the need for such research but that the number of investigators that could be supported was diminishing. It is significant that this situation was recognized in part by Congressional appropriations committees when they increased the 1955 appropriations for dental research to $421,000. During 1953 and 1954, this phenomenon of decreasing numbers of grantees receiving the $221,000 appropriation pointed up the conditions in dental-sponsored and identified research that were leading dental schools to widespread criticism of the dental research grants program. Many investigators complained that the program tended to perpetuate grant support to a few favored investigators and institutions. Although the admission was readily forthcoming from complainants that the investigators and institutions getting the money were generally the best qualified, they believed that the concentration of grant funds in a few research centers prevented the entry of "new blood" into dental research and the exploration of new problems and broader areas of interest. The increased appropriation of $421,000 in fiscal 1955 helped to allay this situation, but its benefit was felt for only that one year.

The Dental Council took the problem under consideration and appointed an ad hoc Program Planning Committee. During calendar year 1955, members of this committee visited all dental schools to discuss the grants program. On the basis of the findings of this committee, it is hoped that our grants program can be broadened in scope and that research can be encouraged on a limited scale in schools not presently participating in the program.

RESEARCH FELLOWSHIPS:

Although the present research potential available in this country is not being used, the replacement of this potential and its expansion are paramount. The fellowship program is designed to cope with this situation. The inauguration of the dental students part-time research program has been a major step forward in this direction. This program as designed insures an increasing number of applicants for graduate training in preparation for research careers.

R.P.C. - 1
December 1955 Analysis of NIH Program Activities

Project Description Sheet

1. Dental Research
INSTITUTE

2. Oral and Biological Chemistry
LABORATORY

3. --

4. --

5. NIDR - 1
SERIAL NO.

6. Dental Caries - Nutrition and Biochemistry
PROJECT TITLE

7. F. J. McClure, L. A. Bavetta, I. Zipkin, R. Harris, J. E. Folk
PRINCIPAL INVESTIGATORS

8. H. Fullmer
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: (a) to evaluate dietary factors associated with dental caries, specifically to investigate the relation of heat processing of foods (cereals and milk powders) to the production of experimental dental caries. (b) to investigate the role of lysine and its relation to skeletal and dental tissue histology. (c) to observe systemic effects, other than the teeth, which may be identified with dental caries. (d) to study caries potentiating and inhibiting agents, particularly EDTA and DHA and ascertain their mode of action.

Methods Employed: Experimental caries is produced in the white rat by various diets and factors influencing this caries development are studied. Chemical substances studied for this effect on caries development were dehydroacetic acid, azaguanine, sulfasuxidine and kojic acid. Ethylene diamine tetraacetic acid is being studied especially for its potentiating effect on caries as well as its systemic effect on the animal. It is becoming increasingly clear that the heat processing of food proteins may result not only in a reduced availability of the essential amino acid lysine, but also of other nutritional factors. The role of minerals may be an important associated factor. The conditions influencing changes produced by heat processing of foods are studied for their effects on the cariogenic potential of the processed food, and in addition the basic chemical nature of the sugar-protein reaction occurring during heat-processing is studied.

Lysine deficiency studies on experimental rats are carried on using diets with zein as the base of inadequate protein. In addition diets containing casein-lactose mixtures autoclaved under varying conditions are studied in an attempt to pin point the nature, as well as the conditions which may catalyze the

Methods Employed (Continued):

destructive effects of heat on casein. The histology of typical long bones, alveolar bone and dental tissues (as well as other body tissues) are studied in relation to a lysine deficiency.

Major Findings: Caries-potentiating EDTA, ingested in the diet as well as by intubation, caused a reduction in hemoglobin. Pathological lesions were found in the liver and kidney apparently due to EDTA.

An extended study of the relation of lysine to the production of caries by heat processed foods has furnished substantial evidence of a pronounced caries inhibitory effect of lysine in certain lysine deficient diets.

The intubation of lysine as well as its ingestion in drinking water have furnished interesting evidence of caries-inhibiting activity. If it may be proved that lysine actually can by-pass the oral cavity, and at the same time inhibit caries, the finding will have significance relating to systemic factors and dental caries etiology.

Smooth surface caries identical with that resulting from heat processed skim milk powders has resulted also from inadequate diets containing an excessive quantity of whole wheat flour.

Sodium laurol sarcosinate had a significant inhibitory effect whereas dehydroacetic acid had a potentiating effect on smooth surface caries. The mode of action of the latter may be related to systemic factors outside the oral cavity.

Significance to Dental Research: The results contribute to knowledge of the etiology of dental caries and particularly to the role of diets which contain foods which have undergone severe heat processing. Studies on agents which either inhibit or promote dental caries contribute to knowledge of the etiology of caries. Systemic response of animal to these agents, as well as systemic changes associated with dental caries, broadens the over-all "picture" of caries as a disease related to other parts of the body.

Proposed Course of Project: Work will continue along present lines with emphasis on use of more completely synthetic or purified diets. Likewise the role of lysine will be more thoroughly studied particularly for its effects systemically and its relation to pathology of dental and calcified structures. The effect of other amino acid deficiences will be investigated. In addition to skim milk powders, typical cereal foods such as whole wheat flour will be studied for further information on the effects of heat processing.

R.P.C. - 3
December 1955

BUDGET DATA

10. NIDR - 1
SERIAL NO.

11. --

12. BUDGET ACTIVITY: Research

13. IDENTIFY ANY COOPERATING UNITS: Dr. Martin Rubin of Georgetown University is an advisor in part of this project.

14. None

HONORS, AWARDS, AND PUBLICATIONS

15. --

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

McClure, F. J. and Folk, J. E. Lysine and Cariogenicity o of Two Experimental Rat Diets. Science 122:557, 1955.

McClure, F. J. and Folk, J. E. Experimental Variables Affecting Caries Production in Rats Fed Diets Containing Processed Foods. J. Dent. Res. 34:358, 1955.

McClure, F. J. Dietary Factors in Experimental Rat Caries. Chapter in Advances in Experimental Caries Research published by AAAS, 1955, pp. 107-124.

McClure, F. J. and Folk, J. E. Observations on the Production of Smooth Surface Rat Caries by Diets Containing Skim Milk and Whey Powders. J. Nutrition 55:589, 1955.

Lossee, F. L. and McClure, F. J. The Dental Caries and Rate of Growth in Three Strains of White Rats. Naval Med. Res. Institute Research Reports Vol. 13, pp. 197-206.

McClure, F. J, Folk, J. E. and Rust, J. D. Smooth Surface Caries in White Rats: Effect of Fluoride, Iodoacetate, Penicillin, Crisco, Butterfat and a Salt Mixture. J. Am. Dental Assoc. In Press.

Zipkin, I. and McClure, F. J. The Effect of Sodium Laurol Sarcosinate and Dehydroacetic Acid on Sulcal and Smooth Surface Caries in the White Rat. J. Am. Dental Assoc. In Press.

17. None

R.P.C. - 1
December 1955 Analysis of NIH Program Activities

Project Description Sheet

1. Dental Research
INSTITUTE

2. Oral and Biological Chemistry
LABORATORY

3. --

4. --

5. NIDR - 2
SERIAL NO.

6. Dental caries in the Syrian hamster
PROJECT TITLE

7. P. H. Keyes
PRINCIPAL INVESTIGATOR

8. None

9. PROJECT DESCRIPTION:

Objectives: To identify and propagate strains of hamsters susceptible to dental caries. To develop, if feasible, inbred strains of such animals. To use these animals and strains to investigate factors associated with caries activity.

Methods Employed: Animals are selected on the basis of known susceptibility. These animals are inmated or cross-mated. All animals are raised under laboratory conditions which are carefully standardized with respect to light cycle, temperature, diet, weaning time, etc. Following weaning, animals are placed on a highly cariogenic diet for 6 to 8 weeks. Examinations are made of the live animal at various intervals to ascertain progress of carious lesions. After 8-10 weeks the most susceptible animals are saved for further propagation. Others are killed.

Major Findings: The major finding of this project during the past ½ year has been the finding of animals which develop rampant dental caries after 6-8 weeks on the test diet used. Gross incipient cavitation of enamel has been observed after 10-12 days on the test diet. Whole molar destruction is occasionally seen between 5-6 weeks.

Significance to Dental Research: In the study of local and systemic factors associated with dental caries, animals must be susceptible to the disease. With the development of the present line of hamsters, it should be possible to study the effect of caries inhibitory agents; nutritional factors either pre-eruptively, post-eruptively, or both; bacteriological factors during the progress of lesion development, etc.

Proposed Course of Project: During the next year an effort will be made to reduce the number of lines of hamsters (approx. 50) to several of the most susceptible. Second generations are now being tested. Some information on third and fourth generations should be available by the latter part of the year. Production should reach the level of approximately 100 per month. Testing procedures will be improved, if possible. This involves the determining the best time interval for examining the animals as well as the best dietary regimens. Other types of assays will be conducted if possible.

BUDGET DATA

10. --

11. --

12. BUDGET ACTIVITY: Research

13. - 14. None

HONORS, AWARDS, AND PUBLICATIONS

15. - 17. None

R.P.C. - 1
December 1955 Analysis of NIH Program Activities

Project Description Sheet

1 Dental Research
INSTITUTE

2. Oral and Biological Chemistry
LABORATORY

3. --

4. --

5. NIDR - 3
SERIAL NO.

6. Inorganic Chemistry of Teeth
PROJECT TITLE

7. H. G. McCann
PRINCIPAL INVESTIGATORS

8. R. C. Likins
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To find what reactions take place between mineralized tissues and certain other substances such as fluoride which offer some protection to the teeth against dental caries.

Methods Employed: Reactions of fluorides have been determined in vitro by analysis of products and by-products of the reactions and in vivo by analysis of the products and comparison with in vitro products. Effect of fluoride on phosphorus exchange has been studied with radioactive phosphorus.

Major Findings: During the past year it has been shown that the material formed by the in vitro reaction of fluoride ions with dentin and bone is probably produced by ionic exchange and diffusion, and is very similar to the material produced by the in vivo reaction with fluoride. However, when enamel is treated in vitro the reaction takes place mostly on the outer surfaces probably because of the extremely slow thermal aging of enamel as appeared to bone and dentin, but fluoride is incorporated throughout the tissue in the in vivo reaction. The in vivo reaction product of fluoride with the mineralized tissues of the rat is almost surely fluorapatite.

The rates of surface exchange and the subsequent slower exchange of phosphorus by diffusion were increased in the presence of 2 or 10 ppm fluoride in the buffer. The rate of the initial exchange was faster in most of the samples already containing fluoride by incorporation either in vivo or in vitro but the rate of the subsequent exchange was decreased, probably because of decreased solubility. Both of these rates were increased by the presence of fluoride in the buffer. A number of analyses, ash, calcium, phosphorus, magnesium, carbon dioxide on human autopsy material, were completed.

Significance to Dental Research: The more that can be learned about the reactions and composition of mineralized tissues that influence dental caries, the sooner the use of less empirical methods may be employed in the prevention of this disease.

Proposed Course of Project: A study is now being made of the reaction of stannous fluoride with mineralized tissues. It is planned to investigate the effect of changes in composition of the teeth on dental caries by attempting to change the composition of the teeth of experimental animals by diet. It is also proposed to investigate further the composition of various treated and untreated mineralized tissues by means of high temperature, ashing and by study of pyrophosphate formation on ashing. Additional study of synthetic hydroxyapatite by means of radioactive Ca and P is also proposed.

BUDGET DATA

10. --　　11. --

12. BUDGET ACTIVITY: Research　　13 - 14. None

HONORS, AWARDS, AND PUBLICATIONS

15. --

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

McCann, H. G. and Bullock, F. A. Reactions of Fluoride Ion With Powdered Enamel and Dentin. J. Dent. Res. 34:59, 1955.

17. None

R.P.C. - 1
December 1955 Analysis of NIH Program Activities

Project Description Sheet

1. Dental Research
INSTITUTE

2. Oral and Biological Chemistry
LABORATORY

3. —

4. —

5. NIDR - 4
SERIAL NO.

6. Organic Chemistry of Teeth
PROJECT TITLE

7. K. A. Piez and H. L. Wolff
PRINCIPAL INVESTIGATORS

8. J. E. Folk and S. A. Narrod
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: The purpose of this work is to determine the chemical nature of the collagen obtained from dentin.

Methods Employed: Dentin is separated from enamel by flotation; the dentin is decalcified, hydrolyzed, and analyzed quantitatively for amino acids by ion exchange methods.

Major Findings: To the amino acid data previously obtained was added an analysis of cystine employing a specific ion exchange method. The value obtained was 1.6 residues (half cystine) per 10^5 grams of protein. This is the amino acid present in smallest concentration. With this value it was then possible to calculate a minimum molecular weight for dentin collagen. The calculation was based on the assumption that all the molecules in the protein are identical and the fact that the number of residues of any amino acid per protein molecule must be a whole number. The value obtained was 146,000. This is one-half the most recent value obtained from physical measurements, and is, therefore, a reasonable value.

Significance to Dental Research: A knowledge of the basic chemical nature of collagen is a necessary preliminary to studying possible changes in the protein that might occur in dental caries, periodontal disease, and other conditions.

9. PROJECT DESCRIPTION (Continued):

Proposed Course of Project: This phase is complete. It is planned to study the proteins of enamel matrix and enamel cuticle.

BUDGET DATA

10. --

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12. BUDGET ACTIVITY: Research

13 - 14. None

HONORS, AWARDS, AND PUBLICATIONS

15 - 17. None

R.P.C. - 1
December 1955 Analysis of NIH Program Activities

Project Description Sheet

1. Dental Research
INSTITUTE

2. Oral and Biological Chemistry
LABORATORY

3. --

4. --

5. NIDR - 5
SERIAL NO.

6. Chemistry and Physiology of Salivary Secretion.
PROJECT TITLE

7. I. Zipkin, K. A. Piez, H. L. Wolff
PRINCIPAL INVESTIGATOR(S)

8. None
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION:

Objectives: (1) To determine which amino acids are normally present in pure parotid and submaxillary secretions of normal human subjects, and the concentrations of these amino acids; (2) to determine the effect of ingestion of large quantities of these amino acids on their concentration in pure salivary secretions; and (3) to establish definitely the structure of an "unusual" amino acid in whole saliva which is probably δ-amino valeric acid.

Methods Employed: Lashley cups and a modified Schneyer device are used to collect the pure parotid and submaxillary secretions respectively. Dialysis and precipitation are used for deproteinization; paper chromatography and ion-exchange chromatography are used to identify the amino acids and establish their concentrations.

Major Findings: The free amino acids so far tentatively identified in pure parotid saliva are taurine, lysine, aspartic acid, glutamic acid, arginine, glycine, serine and alanine. Their concentrations appear to be in the order of one or two µM per liter of saliva. This is about one-tenth to one-thousandth of the concentration found by other investigators for these amino acids in whole salive. Citric and lactic acids have been qualitatively identified in whole saliva.

Significance to Dental Research: While the literature contains a number of publications concerning free amino acids in saliva, none of the work was done on pure salivary secretions. This has resulted in a great deal of disagreement, in regard to both the quantitative and qualitative aspects of the subject. This work should help to show the origin of the free amino acids found in the oral cavity, including δ-amino valeric acid.

9. PROJECT DESCRIPTION (Continued):

Proposed Course of Project: During the next year the work already done on parotid saliva will be repeated on submaxillary saliva; this work will also be extended to obtain quantitative values. The "unusual" amino acid in saliva will be compared to δ-amino valeric acid in an effort to prove their identity. Some attempt will be made to investigate factors involved in the concentration levels of the amino acids in pure salivary secretions. Work on identification of organic acids in the various salivary secretions is being pursued using various methods of preparation of samples. Lysine content of saliva will be investigated initially.

BUDGET DATA

10. --

11. --

12. BUDGET ACTIVITY: Research

13 - 14. None

HONORS, AWARDS, AND PUBLICATIONS

15 - 17. None

R.P.C. - 1
December 1955 Analysis of NIH Program Activities

Project Description Sheet

1. Dental Research
INSTITUTE

2. Oral and Biological Chemistry
LABORATORY

3. --

4. --

5. NIDR 6
SERIAL NO.

6. Biochemistry and Enzymology of Oral Soft Tissue
PROJECT TITLE

7. Bernard K. Forscher and Stuart Narrod
PRINCIPAL INVESTIGATORS

8. Harold Stanley
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: The objectives of this project are to elucidate the pattern of changes in chemical composition and enzymatic activity which characterize the classic inflammatory response in oral soft tissue and to determine how this pattern is modified by various systemic conditions.

Methods Employed: An inflammatory reaction is generated in the palate of the Sprague-Dawley rat by stimulation with radio frequency current delivered from a standard electrode for exactly one second at controlled current intensities. Tissue samples are taken before and at standard intervals after treatment and are subjected to chemical analysis, enzyme assay and histopathological study. In addition to normal rats, approximately 8 weeks of age, the above procedures are also applied to rats previously subjected to specific systemic treatments.

Major Findings: The findings thus far may be briefly summarized:

1. The severity of the inflammatory lesion is dependent on the intensity of the incident current.

2. The degree of inflammation is closely reproducible.

3. The histological findings indicate that the course of events following stimulation follow the classic picture of inflammation.

4. Significant concentration changes with time following treatment of normal animals have been found for glycogen, total phosphorus, acid soluble phosphorus and deoxyribonucleic acid. Inorganic phosphate remains constant. There are indications of changes in alkaline phosphatase activity.

Major Findings (Continued):

5. Aging, general starvation and lysine-deficient diets reduce the hexosamine content of oral soft tissue in untreated rats. The inflammatory response in old rats (16 months) appears to differ from the younger animals but findings are indefinite as yet.

Significance to Dental Research: Periodontal diseases involving the supporting structures of the teeth cause almost half the tooth loss in this country. This project represents a fundamental approach to the etiology and is based on long standing clinical observations that certain systemic conditions predispose subjects to periodontal disease. Comparisons between the biochemical pattern of inflammatory response in normal tissue and in systemically predisposed tissue may lead to elucidation of the factors that control resistance and susceptibility to inflammation, and, by extension, to periodontal disease. The animal tissue used in these studies is analogous to human gingival tissue in many respects, thus allowing application of these findings to later studies on human material. This work represents the first successful attempt to produce a reproducible standard inflammation suitable for experimental studies on the soft tissues of the periodontal structures.

Proposed Course of Project: The procedure described above will be continued. Chemical analyses will determine changes in hexosamine, tyrosine, hydroxyproline, ribosenucleic acid, total nitrogen, non-protein nitrogen and other tissue components as the need arises. Assay procedures are being devised to follow changes in enzymes of the glycolytic pathway, in proteolytic activity and in acid and alkaline phosphatases in inflamed tissue. When the nature of the acute inflammatory response has been established, an attempt will be made to work out the pattern of chronic inflammation. For the study of effects of systemic conditions on local inflammatory response, it is planned to study animals subjected to dilantin treatment, alloxan diabetes, malnutrition (general and specific), general stress, splenectomy, thyroidectomy, and pretreatments with ACTH, estradiol, progesterone, testosterone and thyroid extract. Chemical analyses, enzyme assays and histologic studies will be carried out on these animals before and after treatment.

BUDGET DATA

10. --
11. --
12. BUDGET ACTIVITY: Research
13 - 14. None

HONORS, AWARDS, AND PUBLICATIONS

15 - 17. None

R.P.C. - 1
December 1955 Analysis of NIH Program Activities

Project Description Sheet

1. Dental Research
INSTITUTE

2. Oral and Biological Chemistry
LABORATORY

3. --

4. --

5. NIDR - 7
SERIAL NO.

6. Protein and Amino Acid Chemistry
PROJECT TITLE

7. J. E. Folk, S. A. Narrod, K. A. Piez, and H. L. Wolff
PRINCIPAL INVESTIGATORS

8. M. S. Burstone, R. R. Omata
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION:

Objectives: The broad purpose of these investigations is to provide information of a fundamental nature concerning the chemistry of amino acids and proteins. Specifically, the past year has seen emphasis placed on (1) the reaction between lysine and sugar, (2) the enzymatic degradation of collagen, (3) the nature of the amino acid pool and some aspects of amino acid metabolism in cells grown in tissue culture, (4) 5-hydroxypipecolic acid, a new imino acid related to hydroxylysine, and (5) the synthesis of amino acid substrates of possible value in the histochemical demonstration of certain peptidases.

Methods Employed: For the study of the lysine-sugar reaction a number of synthetic peptides containing lysine were prepared. The products of the reaction of these compounds with glucose under various storage conditions were investigated with the aid of ion exchange and paper chromatography, paper electrophoresis, N-terminal amino acid determinations, and specific colorimetric reactions.

The degradation of collagen was studied with Clostridium perfringens and with a microorganism isolated from the human oral cavity. The degradation of collagen was followed by the use of several analytical methods designed to measure degradative products. The synthesis of model collagenase substrates has been started.

The amino acid pool and protein of mammalian cells in tissue culture were studied with ion exchange and paper chromatography. In addition, C^{14} tracer methods and paper chromatography were used in the study of amino acid metabolism in these cells.

The imino acid, 5-hydroxypipecolic acid, was investigated by the methods of ion exchange and paper chromatography. Organic

Methods Employed: (Continued)

synthetic methods were used in its preparation from 5-hydroxylysine.

The techniques of organic synthesis were used to prepare amino acid substrates of possible histochemical value.

Major Findings: A small amount of a ε-N-lysine derivative of glucose has been isolated after the storage of lysine and glucose. The same compound was released from the product of the reaction of polylysine with glucose by pancreatic enzymes. Its exact structure is not yet known. The influence of the lysine-glucose combination on the *in vitro* action of various enzymes was determined.

The initial investigations of collagenase were conducted with *Cl. perfringens* and an unknown aerobic oral organism, both of which have been found to produce extracellular collagenase. The enzyme could be precipitated with ammonium sulfate. Its method of action appeared to occur by the release of large peptides rather than free amino acids.

The composition of the free amino acid pool and the total protein of HeLa cells grown in tissue culture has been determined. It was found that both the pool and the protein were maintained essentially unchanged even when the cells were deprived of amino acids essential for growth. With respect to the amino acid metabolism of these cells, it was found that a limited conversion of phenylalanine to tyrosine takes place. Employing C^{14}-labeled glucose and glutamine, it was shown that both compounds could be used for the synthesis of the amino acids known to be nonessential, but were not converted to the nutritionally essential amino acids.

In the course of these studies/on amino acids in cells grown in tissue culture, it was observed that C^{14}-labeling affects the ion exchange behavior of amino acids. The labeled molecules moved somewhat more slowly. Further investigation indicated that the effect was related only to the 1 and 2 positions of the amino acid. A C^{14} atom in a position away from the charge center had no effect on the chromatographic behavior.

The diastereoisomers of 5-hydroxypipecolic acid were separated by ion exchange and paper chromatography. This imino acid was isolated from dates and synthesized *in vitro* from 5-hydroxylysine. In connection with this work a specific colorimetric method for the cyclic imino acids was developed.

Major Findings (Continued):

A number of peptide derivatives of β-naphthylamine were prepared. Their hydrolysis by tissue extracts was studied. It was found that leucyl-β-naphthylamide could be used for the demonstration of aminopeptidases and papain. Prolyl- and hydroxyprolyl-β-naphthylamide were of value in showing imino peptidases. It was found that whole saliva, unlike other tissues, hydrolyzed the hydroxyproline peptide at a rapid rate.

Significance to Dental Research: Inasmuch as enzymes and much structural material of body tissues are protein, studies of these types are basic to most biological problems. The synthesis, degradation and maintenance of proteins and amino acids are one of the most important and fundamental aspects of periodontal disease and dental caries as well as most other diseases. These investigations are contributing to our basic knowledge in these areas.

Proposed Course of Project: It is planned to continue the attempts to identify the exact chemical nature of the lysine-glucose reaction product and to investigate further its effect on amino acid and protein utilization. The methods of studying collagenase action will be expanded and employed in model systems. Further basic studies on amino acid metabolism in tissue culture will be undertaken.

BUDGET DATA

10. NIDR - 7
SERIAL NO.

11. --

12. BUDGET ACTIVITY: Research

13. IDENTIFY ANY COOPERATING UNITS: L. A. Cohen, F. Irreverre, B. Witkop of the National Institute of Arthritis and Metabolis Disease and H. Eagle of the National Microbiological Institute.

14. None

HONORS, AWARDS, AND PUBLICATIONS

15. --

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

Folk, J. E. and Burstone, M. S. Chromogenic Leucyl Substrates for Aminopeptidase and Papain. Proc. Soc. Exptl. Biol. & Med. 89:473, 1955.

16. Continued.

Folk, J. E. Reactions of Glucose with Lysine. Arch. of Biochemistry. In Press.

Cohen, L., Irreverre, F., Piez, K. A., Witkop, B., and Wolff, H. L. Synthesis of 5-Hydroxypipecolic Acid and Separation of its Diasteroisomers. Science. In Press.

Piez, K. A. and Eagle, H. Systematic Effect of C^{14}-labeling on Ion Exchange Chromatography of Amino Acids. Science 122:968, 1955.

Folk, J. E. and Burstone, M. S. The Enzymatic Hydrolysis of Prolyl- and Hydroxyprolyl-naphthylamides. Submitted for publication to Arch. Biochem. and Biophys.

Burstone, M. S. and Folk, J. E. Histochemical Demonstration of Aminopeptidase. J. Histochem. and Cytochem. In Press.

17. None.

R.P.C. - 1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

1. Dental Research
INSTITUTE

2. Oral and Biological Chemistry
LABORATORY

3. --

4. --

5. NIDR - 8
SERIAL NO.

6. Fluorine - Mineral Metabolism, Caries Control and Physiological Effects
PROJECT TITLE

7. R. C. Likins, F. J. McClure and I. Zipkin
PRINCIPAL INVESTIGATORS

8. N. C. Leone, H. McCann
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To obtain information on (a) mineral metabolism of teeth and supporting structures, (b) metabolism of fluorine particularly in calcified tissue and (c) other physiological effects of fluorine relative to its use for the partial control of dental caries.

Methods Employed: Sodium fluoride and radioactive calcium were given young rats to observe effects on tooth formation. The calcium, phosphorus, carbonate and ash content was studied in relation to age of dentin and enamel. The chemistry of dentin and enamel is compared in caries susceptible and caries resistant strains of white rats. Urinary fluorine concentration is studied in relation to fluorine in drinking water. Fluorine analysis is made of human autopsy material in relation to fluorine in drinking water. Metabolism of fluoride is studied in the growing and mature rat.

Major Findings: It has been shown that fluoride can markedly affect calcium exchange in the enamel of rats' teeth. Preliminary results indicate that carbonate in enamel and dentin increases with age. Proportionally more labeled calcium was deposited in the molar enamel of caries susceptible rats, than caries resistant. The results suggest a relation between rate of calcium exchange and caries susceptibility.

The mobilization of fluorine from human skeletal tissue was manifested in the high level of fluoride present in urine specimens of resident of Bartlett, Texas following water defluoridation.

Major Findings (Continued):

The urinary concentration of fluoride associated with the use of fluoridated drinking water was found to equal that associated with the use of natural fluoride water. Young age groups were significantly slower in reaching a stablized relation between urine and water fluoride concentration than individuals who had reached maturity. The concentration of fluoride in human autopsy skeletal tissue associated with prolonged exposure to 8.00 ppm of fluoride in drinking water was ten times higher than the fluoride normally present in skeletal tissues.

Significance to Dental Research: A basic problem which may be solved by this research is the mechanism by which fluorine prevents dental caries. The results contribute information on the relation of fluorine to calcium metabolism. Studies on non-dental effects of fluoride furnish information desirable for the water fluoridation program.

BUDGET DATA

10. -- 11. --

12. BUDGET ACTIVITY: Research 13. - 14. None

HONORS, AWARDS, AND PUBLICATIONS

15. --

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

Zipkin, I., Likins, R. C., McClure, F. J. and Steere, A. C. The Urinary Fluoride Concentration Accompanying Exposure to a Fluoridated Drinking Water. Submitted to Pub. Health Reports.

Zipkin, I and Scow, R. O. Fluoride Deposition in Different Segments of the Tibia of the Young Growing Rat. Am. J. Physiol. In Press.

Likins, R. C., McClure, F. J. and Steere, A. C. The Urinary Excretion of Fluoride Following Defluoridation of a Municipal Water Supply. Pub. Health Reports. In Press.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

F. J. McClure received the Department of Health, Education and Welfare Superior Service Award.

R.P.C. - 1
December 1955 Analysis of NIH Program Activities

Project Description Sheet

1. Dental Research
INSTITUTE

2. Oral Bacteriology
LABORATORY

3. --

4. --

5. NIDR-9
SERIAL NO.

6. The Relationship of Pathogenic Fungi to Diseases of the Oral Cavity. A. *Actinomyces*.
PROJECT TITLE

7. A. Howell, Jr. and L. Pine, NMI
PRINCIPAL INVESTIGATOR

8. None

9. PROJECT DESCRIPTION:

Objectives: To study the incidence and identity of pathogenic or potentially pathogenic fungi in the normal mouth with special emphasis on the filamentous forms of the genus *Actinomyces*; and to study the possible relationship of these organisms to dental caries and to other diseases of the oral cavity or related areas.

Methods Employed: The nutrition of this group of filamentous fungi (*Actinomyces* *Spp.*) has been studied by growing them on various types of artificial culture media under both aerobic and anaerobic conditions. In addition, various generally accepted chemical analyses have been employed for the study of the metabolic products produced by these organisms.

Major Findings: During the past year major emphasis has again been placed on a study of, and metabolic products produced by, filamentous organisms of the genus *Actinomyces*. In addition, a comparison has been made of certain physiological and histochemical characteristics of *Actinomyces* with the same properties of *Lactobacillus* *bifidus*. Thirteen of 15 strains of *Actinomyces*, representing at least two different types of these organisms, have been successfully grown and maintained in a liquid synthetic medium containing starch. Certain strains were found to differ in their response to the pH of the medium; some were found to be anaerobes or microaerophiles, others aerobes or faculative organisms. All strains of *Actinomyces* studied were found to produce lactic, acetic, formic and succinic acids from the fermentation of glucose, with lactic acid being the predominant product.

Cultures of Lactobacillus bifidus were found to be very similar in many respects to cultures of Actinomyces but to differ significantly in their fermentation of glucose.

Significance to Dental Research: As pointed out previously, numerous reports may be found in the literature concerning the presence of filamentous organisms in the oral cavity or adjacent areas. However, in the majority of instances complete descriptions of the organisms isolated on specific designations have not been given so that the true incidence and importance of such organisms in the oral flora is still open to question. The successful cultivation of these organisms on a medium of known composition will permit critical studies of the growth requirements of these forms. Conclusive evidence that some strains of Actinomyces differ markedly in their oxygen tolerance will no doubt when fully elaborated, lend substantial assistance to species differentiation and hence permit a more accurate estimate of the incidence of the various forms in the oral cavity and related regions and of the possible role(s) they may play in the ecology of diseases of these areas.

Proposed Course of Project: During the coming year, tentative plans call for (a) a more thorough study of the oxygen requirements on tolerance of strains of Actinomyces; (b) a study of their nutritional requirements; and (c) possibly the initiation of studies on the incidence of these forms in the oral cavity.

BUDGET DATA

10. --

11. --

12. BUDGET ACTIVITY: Research

13. IDENTIFY ANY COOPERATING UNITS: Laboratory of Infectious Diseases, National Microbiological Institute for both 1955 and 1956.

14. None

HONORS, AWARDS, AND PUBLICATIONS

15. --

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

Howell, Jr., A. and Pine, L.: Studies on the Growth of Species of Actinomyces. I. Cultivation in a Synthetic Medium with Starch. (In press).

17. None.

R.P.C. - 1
December 1955 Analysis of NIH Program Activities

Project Description Sheet

1. Dental Research
INSTITUTE

2. Oral Bacteriology
LABORATORY

3. --

4. --

5. NIDR-10
SERIAL NO.

6. Studies on Latent Virus Infections.
PROJECT TITLE

7. W. D. McBride
PRINCIPAL INVESTIGATOR

8. None

9. PROJECT DESCRIPTION:

Objectives: To develop a system in which one can study the host-parasite relations of latent virus infections under carefully controlled conditions.

Methods Employed: (a) Attempts are being made to establish a lysogenic type infection of human or animal viruses in established mammalian cell lines. This is being done by establishing conditions which select mutant cells capable of propagating provirus or viruses capable of being propagated in a provirus state.

(b) Different technics are employed to induce cells which are believed to be latently infected to produce virulent virus.

(c) Animals are inoculated with viruses thought to produce latent infections and the survivors of such inoculations are used as a source of cells for culture after the clinical infection has subsided. Attempts are made to demonstrate the presence of virus or provirus in such cells.

(d) Technics of single cell isolation, infection, and harvests for virus titer by the plaque technic are used to determine the nutritive requirements of virulent and provirus development. Observations with the phase and darkfield microscopes are correlated with virus development within and release from the cells.

Major Findings: Three lines of Hela cells were developed which continued to produce poliomyelitis virus over a period of several months. Various means were employed in testing whether or not the infection was analogous to lysogeny in phage or rather like the carrier state of phage. The cells were lost due to contamination before the question was resolved. This work is being repeated.

A paper is in process of preparation on the virus susceptibility of a normal strain of human skin cells, #1769 of Dr. Wilton Earle.

Significance to Dental Research: There are numerous instances of human and animal infections which are thought to be latent in some phase of their existence. By latent, I refer to the lysogenic type infection of phage and not an incubating, abberant, quiescent, or residual low grade infection--all conditions confused in terminology in the literature. In addition, it has long been suggested and bolstered with experimental results from work done on lower animals, that cancer may be caused by a latent virus infection.

There has been little revealing work done in the field of latent infections due to the lack of adequate technics. Now that most of the procedures long available for phage research have been adapted to animal viruses and cells, the possibility exists for much fundamental research being accomplished on this special type of host-parasite relationship. It is felt that the development and study of a latent infection in tissue culture would lead to technics whereby much new information may be gained. The importance of such information to the field of infectious diseases as well as cancer and general cellular growth and development could be enormous.

Proposed Course of Project: Efforts will continue along lines outlined under methods (above).

BUDGET DATA

10. --

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12. BUDGET ACTIVITY: Research

13. - 14. None

HONORS, AWARDS, AND PUBLICATIONS

15. --

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

Habel, Gregg, and McBride. Susceptibility to poliomyelitis virus of Hela cells adapted to growth in horse serum medium. Soc. Exp. Biol. & Med., 92:87, 1955.

17. None

R.P.C. - 1
December 1955 Analysis of NIH Program Activities

Project Description Sheet

1. Dental Research
INSTITUTE

2. Oral Bacteriology
LABORATORY

3. --

4. --

5. NIDR-11
SERIAL NO.

6. Ulcerative Gingivo Stomatitis: The Significance of the Oral Spirochetal Flora in Etiology of Certain Ulcerative Lesions of the Oral Cavity.
PROJECT TITLE

7. E. G. Hampp
PRINCIPAL INVESTIGATOR

8. None.

9. PROJECT DESCRIPTION:

Objective: The development of routine laboratory technics for the isolation and pure cultivation of the oral spirochetes; develop methods for classification and identification of these various microorganisms on a sound basis, and to determine their etiologic significance to the disease.

Methods Employed: Although all the recognized morphologic types of oral spirochetes have been obtained in pure culture as the result of these studies, a routine technic for laboratory use has not been perfected for *Borrelia buccale*. The methods employed utilize a Wellplate Method developed in this laboratory for separation of the oral spirochetes from oral contaminants with final separation of the spirochetal components by shake tube methods. A specialized medium has been developed for the above purpose. Inocula for culture material has been obtained from patients in the Clinical Center.

Major Findings: It has been found that about 80% primary culture plates contain *B. buccale* along with the other oral spirochetes; however it is in the shake tube procedure for isolation of individual microorganisms that the technic falls down. Various nutriments have been added to the basic medium for improving this phase of the technic, some success has been encountered but further studies are required to resolve the problem.

It has become increasingly apparent that it is essential to develop a synthetic medium by working out the growth requirements of these little understood group of microorganisms. This is necessary in order to develop a routine method for isolation of *B. buccale* as well as conduct biochemical and serologic studies as they relate to classification and etiology in this problem.

Further attempts are being made to preserve stock strains of B. buccale by freeze-drying procedures as developed for the other types of oral spirochetes isolated in these investigations. A small amount of success has been encountered in this respect as evidenced by an occasional pure culture or organisms surviving these procedures.

Further studies are in progress in this respect.

Significance to Dental Research: These investigations will contribute to our knowledge and understanding of the oral bacterial flora of man and the possible relationship of these organisms to certain types of oral ulcerative lesions. Further, any information gained from these investigations may possibly be applicable to the study of known pathogenic spirochetes peculiar to man such as Treponema pallidum. In this respect, attempts are being made to isolate and cultivate T. pallidum by the methods employed for the oral spirochetes. Some success has been encountered as evidenced by survival of these organisms up to 12 days on our media.

Proposed Course of Project: A considerable portion of our time during the next year will be devoted to the development of a routine technic for isolation of B. buccale and the investigation of the growth requirements of all the oral spirochetes with the view of developing a synthetic medium to be employed for biochemical, serologic and animal studies.

BUDGET DATA

10. - 11. --

12. BUDGET ACTIVITY: Research

13. - 14. None

HONORS, AWARDS, AND PUBLICATIONS

15. - 17. None.

R.P.C. - 1
December 1955 Analysis of NIH Program Activities

Project Description Sheet

1. Dental Research
INSTITUTE

2. Oral Bacteriology
LABORATORY

3. --

4. --

5. NIDR-12
SERIAL NO.

6. Investigation of the Spirochetal Organisms of Non-exposed Non-vital Pulps of Human Teeth.
PROJECT TITLE

7. E. G. Hampp
PRINCIPAL INVESTIGATOR

8. --

9. PROJECT DESCRIPTION:

Objectives: To determine the mode of entrance of such organisms into the pulp of teeth and to determine their role in the initiation and progress of such infections. Further, to study these organisms and compare them both biochemically and serologically with stock strains of oral spirochetes.

Methods Employed: Infected non-exposed (by caries) pulps are sampled by placing a rubber dam in place around several teeth. Using sterile procedures the tooth is opened, the pulp exposed and samples are removed for cultural procedures employing the technics developed in this laboratory for the oral spirochetes. Only about 16 patients have been used thus far since difficulties are experienced in obtaining infected root canals that are not exposed by dental caries.

Major Findings: Of the sixteen patients studied, 8 strains of spirochetes have been isolated in pure culture; 3 strains of organisms were grown in mixed cultures and were never obtained pure. Even though these organisms appear to be morphologically similar to the small oral treponemes, they vary considerably in respect to culture characteristics and in addition, two strains produce gas which has not been encountered heretofore in the organisms derived from the oral cavity. Attempts have been made to develop a sugar free broth for biochemical studies of these organisms and compare them with similar morphologic types obtained from the mouth of human subjects. Such attempts to develop a suitable broth for this purpose have been unsuccessful, which further substantiates the need for a synthetic medium. One of the most intriguing phases of this problem is the mode of entrance of these microorganisms into the pulp without exposure of the pulp by dental caries. It appears therefore that these organisms may possess invasive qualities which is of considerable importance since it has never been conclusively demonstrated that oral spirochetes are potential pathogens.

Significance to Dental Research: These investigations may contribute to our fundamental knowledge concerning the classification, pathogenicity and ecology of these organisms.

Proposed Course of Project: To develop synthetic media to be employed in cultivating these organisms for biochemical, serologic and animal studies. To determine the mode of entry of these organisms to non-exposed pulps of teeth and to determine whether or not they are pathogenic. It is further proposed to investigate the periapical tissues and granulomas occurring at the apex of such teeth by cultural and histologic methods to determine whether these organisms may invade and affect periapical tissues.

BUDGET DATA

10. --
11. --
12. BUDGET ACTIVITY; Research
13. IDENTIFY ANY COOPERATING UNITS: Naval Dental School, National Naval Medical Center, Bethesda, Md.
14. None.

HONORS, AWARDS, AND PUBLICATIONS

15. --
16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

Geo. C. Paffenbarger, J. A. English, E. G. Hampp. Dental Research--Current and Future. J. Am. College of Dentists, 22:21-29, June 1955.

17. None.

R.P.C. - 1
December 1955 Analysis of NIH Program Activities

Project Description Sheet

1. Dental Research
INSTITUTE

2. Oral Bacteriology
LABORATORY

3. --

4. --

5. NIDR-13
SERIAL NO.

6. Study of the Interrelationships Between Local and Systemic Factors in the Etiology of Dental Caries.
PROJECT TITLE

7. R. M. Stephan
PRINCIPAL INVESTIGATOR

8. None

9. PROJECT DESCRIPTION:

Objectives: The objectives of this project are: (a) To investigate experimental conditions which are essential for the development of carious lesions in laboratory animals; (b) To further develop methods for studying the localized formation of bacterial plaques on teeth, the pH of plaques and caries activity *in vivo*; (c) To further explore the relationship which exists between systemic factors and the development of dental caries.

Methods Employed: Experiments have been conducted using 4 stock strains and 10 highly inbred strains of rats. A few experiments with different strains of hamsters and mice have been included for comparative purposes. Different types of diets, including synthetic diets, have been used to induce the different types of carious lesions. Direct *in vivo* microscopic observations of developing carious lesions and pH measurements of bacterial plaques have been made on the incisor teeth of rats. Quantitative determinations have been made of lactobacilli in plaque material removed from specific areas on these teeth. Vital stains have been employed as indicators to delineate the earliest stages of caries.

Major Findings: The unexpected finding of carious lesions in the incisor teeth of a few laboratory rats was reported last year. We have now succeeded in producing these lesions in a large group of animals on an experimental basis. The incisor teeth have an important advantage over the molar teeth of rats in that they permit the study of the effects of post-weaning factors which affect tooth formation on the susceptibility of teeth to caries.

Studies of the pH of bacterial plaques on the incisor teeth of rats by electrometric and colorimetric indicators showed that acidity on these teeth was localized to very small areas, and that the smallest available pH electrodes were still not small enough to permit measurements confined to carious areas only. In general the pH of bacterial plaques and saliva in rats' mouths was around one pH unit higher than that in humans.

Cultures from bacterial plaques on incisor teeth of rats showed no lactobacilli, whereas swabs from the entire mouth of the same rats gave positive cultures for lactobacilli. This finding demonstrates the need for sampling bacterial plaques from specific areas on the tooth surface in order to study the bacteria which are associated with caries.

The _in vivo_ use of alizarin was introduced for demonstrating carious lesions at their earliest stages.

Significance to Dental Research: The development of carious lesions in the incisor teeth of rats provides an experimental basis for studying the effects of systemic factors which affect tooth formation together with local factors which activate the caries process. The multifactorial approach comparing different strains of rats, different diets, and different types of carious lesions, furnishes a broad basis for determining the interrelationship between different factors in the etiology of caries.

Proposed Course of Project: Previous experiments on this and other projects have indicated that many dietary and nutritional factors which affect growth and the general systemic condition may also affect the development of caries in rats. It is planned to concentrate on finding out how some of these systemic factors affect the localized conditions on tooth surfaces when carious lesions develop. Special attention will be directed to the continuously forming incisor teeth because of the possibility of demonstrating the effect of tooth formative factors in them, and also because of their greater accessibility for _in vivo_ tests than the molar teeth. These tests will include direct microscopic observations of tooth surfaces for the development of carious lesions, direct pH measurements on tooth surfaces and the use of indicators and vital stains, and the selective removal of plaque material for bacteriological studies.

It is also planned to continue the study of dental diseases in animals from experiments on specific nutritional deficiencies which were secured from investigators in other Institutes.

Analysis of NIH Program Activities

Budget Data Sheet

10. NIDR-13
SERIAL NO.

11. --

12. BUDGET ACTIVITY: Research

13. IDENTIFY ANY COOPERATING UNITS: A number of investigators from other Institutes of NIH have furnished material from their animal experiments for study of dental diseases in these animals.

14. None

HONORS, AWARDS, AND PUBLICATIONS

15. --

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

Chapter entitled "Location of Experimental Caries on Different Tooth Surfaces in the Norway Rat." Robert M. Stephan and M. Rachel Harris. Published in "Advances in Experimental Caries Research." Monograph published by the American Association for the Advancement of Science. 1955.

17. None

R.P.C. - 1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

1. Dental Research
INSTITUTE

2. Oral Bacteriology
LABORATORY

3. --

4. --

5. NIDR-14
SERIAL NO.

6. The Occurrence of Periodontal Disease and Dental Calculus Formation in Laboratory Rats.
PROJECT TITLE

7. R. M. Stephan
PRINCIPAL INVESTIGATOR

8. None

9. PROJECT DESCRIPTION:

Objectives: The objective of this project is to determine the factors which are associated with the development of periodontal disease in laboratory rats.

Methods Employed: In studies on dental caries in rats, we have observed and recorded varying degrees of periodontal disease, including extensive destruction of alveolar bone, mechanical impaction of food and debris, bacterial plaques and calculus formation. The history of the animals, including the strain, diet, weight curves, and in some cases autopsy data, are recorded, and correlations are being made.

Major Findings: Preliminary analysis of the data indicates that generalized susceptibility of rats to periodontal disease is associated with dietary deficiencies and poor growth of the animals, while excessive localized destruction of periodontal tissue is associated with localized impaction of food and debris. Bacterial plaque formation on teeth appears very extensive in periodontal disease, but hard dental calculus deposits have been found to form without associated periodontal disease.

Significance to Dental Research: Very little is known about the etiology of periodontal disease in humans. These animal studies furnish a valuable basis for the experimental study of various factors which may either increase or decrease susceptibility to this disease.

Proposed Course of Project: It is planned to continue this study, and it will be very worthwhile to conduct more extensive studies on this project if additional personnel become available.

Analysis of NIH Program Activities

Project Description Sheet

10. NIDR-14
SERIAL NO.

11. --

12. BUDGET ACTIVITY: Research

13. IDENTIFY ANY COOPERATING UNITS: A number of other investigators from other Institutes at NIH have furnished material from their animal experiments for study of the dental diseases in these animals.

14. None

HONORS, AWARDS, AND PUBLICATIONS

15. - 17. None.

R.P.C. - 1
December 1955 Analysis of NIH Program Activities

Project Description Sheet

1. Dental Research
INSTITUTE

2. Oral Bacteriology
LABORATORY

3. --

4. --

5. NIDR-15
SERIAL NO.

6. Studies on the Fusobacteria. Nutritional Requirements and the Development of a Selective Culture Medium for Oral Fusobacteria.
PROJECT TITLE

7. R. R. Omata
PRINCIPAL INVESTIGATOR

8. --

9. PROJECT DESCRIPTION

Objectives: The objectives of this project are to study the physiology and taxonomy of the oral fusobacteria, and to develop bacteriologic media for their selective isolation and enumeration.

Methods Employed: The nutritional requirements of the fusobacteria are being studied with the use of a chemically defined medium. Certain growth factors and antimetabolites are also employed to elucidate the metabolism of fusobacteria and to differentiate these organisms from similar types of bacteria.

A new culture medium is being developed for the selective isolation of fusobacteria. This medium is rendered highly selective by the use of bacteriostatic agents, such as crystal violet and streptomycin which inhibit the growth of concomitant bacteria.

Major Findings: It has been found that pantothenic acid is an essential vitamin for growth of fusobacteria whereas other vitamins may be stimulatory but not actually required for growth. The growth of fusobacteria is stimulated by the presence of adenine and hypoxanthine in the culture medium. It is of special interest that a precursor of purine in the form of 4-amino 5-imidazole carboxamide has also been found to be stimulatory.

A highly satisfactory culture medium, containing streptomycin and crystal violet as bacteriostatic agents, has been developed for the selective isolation of oral fusobacteria from human saliva samples.

Significance to Dental Research: In order to study the role of the bacterial flora in relation to oral diseases it is necessary that basic information concerning the physiology of the various types of bacteria be obtained. With the accumulation of such knowledge, it will be possible to learn more about the ecological role of each type of bacteria and what bearing the microorganisms have on the host-parasite relationship, not only in normal subjects but also in pathological conditions. It is important to develop various methods of analyses and culture media to isolate and classify the myriad of microbial forms found in the oral cavity. Many of the bacteriologic methods, especially the less known forms such as the fusobacteria, have been inadequate for quantitative and diagnostic procedures. Since the fusobacteria have been implicated in different oral conditions, i. e. periodontal diseases and Vincent's infection, it is important to obtain basic and fundamental information about them as well as other bacteria.

Proposed Course of Project: It has not been possible to get all the known types of fusobacteria to grow on presently available media. With some of the knowledge thus far accumulated it will be possible to explore further the nutritional requirements of the different species and also to study the metabolic activity of the organisms in respect to amino acids and other nitrogen compounds. Studies will be continued to improve the selective medium and also to isolate and enumerate fusobacteria in humans and experimental animals, normals and those with periodontal and other pathological conditions.

BUDGET DATA

10. --

11. --

12. BUDGET ACTIVITY: Research

13. - 14. None

HONORS, AWARDS, AND PUBLICATIONS

15. - 17. None

R.P.C. - 1
December 1955 Analysis of NIH Program Activities

Project Description Sheet

1. Dental Research
INSTITUTE

2. Oral Bacteriology
LABORATORY

3. --

4. --

5. NIDR-16
SERIAL NO.

6. Studies on the Ecology of the Oral Microflora
PROJECT TITLE

7. M. Rogosa
PRINCIPAL INVESTIGATOR

8. R. J. Fitzgerald, T. Shiota, H. V. Jordan

9. PROJECT DESCRIPTION:

Objectives: (1) To determine the biological distribution of the total oral flora in health and disease, particularly the strongly acidogenic and acid-consuming microorganisms, and (2) to assess the role of the oral microflora in relation to oral diseases such as dental caries and periodontal diseases. This assessment depends largely on a proper understanding of the physiology of these bacteria under various conditions. The following major problems are under study:

(a) Taxonomic studies which include quantitative biochemical studies on the formation of end-products during the biochemical attack on available substrates.

(b) Study of the changes in the oral flora of the hamster and rat which occur with the development of caries.

(c) Study on the utilization of lactic and the organic acids in the oral environment.

Methods Employed: Animal experimentation using cariogenic and non-cariogenic diets; sampling and culturing of oral samples on selective media developed for this project; pure culture studies using biochemical and other analytical methods; use of nutritional test media to determine nutritional requirements of microorganisms; use of Warburg technique, etc.

Major Findings: (1) The diptheroid-like organisms were discovered to be the most numerous group in the oral cavity of the rat. Methods were developed for their selective cultivation.

(2) It was found that previous methods for the cultivation of oral streptococci from the rat were inadequate. An optimal medium was designed for their selective isolation.

(3) A highly selective optimal medium for the isolation and enumeration of the Veillonella was developed. A manuscript is being prepared for formal publication.

(4) A cultural and biochemical study of 500 strains of oral bacteria from rats maintained on a cariogenic diet was completed. The organisms comprised chiefly streptococci, diptheroids, Veillonella, bacteroids, and micrococci. In addition to the usual biochemical and cultural tests the fermentative capacity of the strains was determined on a large number of carbohydrate substrates. These data are now being evaluated.

Significance to Dental Research: It is now generally agreed that microorganisms are involved in the development of dental caries. How they are concerned is the problem. The formation of acid seems necessary for the initiation of dental decay. However, information on the dissipation of acids is almost completely lacking. Since it is highly likely that there is an active competition between the production and consumption of organic acids by different groups of organisms, it is obvious that a beginning must be made in the study of the utilization of organic acids by the oral flora.

Proposed Course of Project: During the coming year work is to be continued to gain a knowledge of the nutrition and biochemistry of the Veillonella. A study of the bacteroids has been initiated. A study of the nutrition and physiology of the diptheroids will be started.

BUDGET DATA

10. -- 11. --

12. BUDGET ACTIVITY: Research

13. IDENTIFY ANY COOPERATING UNITS: American Dental Association (Dr. Shiota)

14. --

HONORS, AWARDS, PUBLICATIONS

15. - 16. None.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955: Elected to executive council of the Washington Branch of the Society of American Bacteriologists. Invited to serve as consultant on research on the lactobacilli by the National Institute of Research for Dairying of England.

Project Description Sheet

1. Dental Research INSTITUTE	2. Oral Bacteriology LABORATORY

3. --

4. --

5. NIDR-17
SERIAL NO.

6. Effect of Skim Milk Powder Diets on Oral Bacterial Flora of Rats and Relation to Experimental Dental Caries.
PROJECT TITLE

7. T. Shiota PRINCIPAL INVESTIGATOR	8. M. Rogosa and F. J. McClure OTHER INVESTIGATORS

9. PROJECT DESCRIPTION:

Objectives: The objective of this project is to determine the effect of skim milk powder diets on microbial oral flora with specific reference to the knowledge of associative and/or causative microorganism(s) of experimental dental caries.

Methods Employed: Weanling Sprague-Dawley rats were placed on a skim milk powder diet (Diet 635), on autoclaved skim milk powder diet (Diet 636), on autoclaved skim milk powder diet supplemented with 2.5% lysine (Diet 636L), and on autoclaved skim milk powder with 0.1% dehydroacetic acid (Diet 636D). Swab samples for bacteriological analyses were taken at zero time, 13th, 34th, 41st, 48th and 62nd days, of the experiment. Caries were evaluated as described by McClure and Folk (Proc. Soc. Expt. Biol. & Med., 83:21 (1953)).

Major Findings: The mean caries score and the mean final lactobacillus counts from rats were respectively on Diet 635, 1.53 and 6,030; Diet 636, 10.13 and 23,540; Diet 636L, 1.89 and 7,920; and Diet 636D, 18.68 and 25,610. Preliminary analyses indicate that none of the other microorganisms studied, diphtheroids, streptococci, Veillonella, and gram negative rods showed a similar trend in their population changes as shown by lactobacilli.

Significance to Dental Research: The lactobacillus population, though low in number as compared to the total oral bacterial population, appeared to reflect the caries experiences in the rats. The populations of other microorganisms though changed with the age of the rats, did not show any relation to the caries.

Proposed Course of Project: It is hoped that with adequate assistance further work to improve the techniques used to study the oral flora can be made. This would be the development and evaluation of the sampling and cultural methods so necessary to conduct the study of the complex oral flora.

BUDGET DATA

10. --

11. --

12. BUDGET ACTIVITY: Research

13. - 14. None.

HONORS, AWARDS, AND PUBLICATIONS

15. - 17. None.

R.P.C - 1
December 1955 Analysis of NIH Program Activities

Project Description Sheet

1. Dental Research
INSTITUTE

2. Oral Bacteriology
LABORATORY

3. --

4. --

5. NIDR-18
SERIAL NO.

6. The Effects of Antibiotics on the Development of Dental Caries.
PROJECT TITLE

7. R. J. Fitzgerald
PRINCIPAL INVESTIGATOR

8. H. V. Jordan and M. D. Lackey
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION:

Objectives: The objectives of this project are: (1) to determine the relative potential of antibiotics as agents for the control of dental caries; (2) to elucidate the role of particular microorganisms in the caries process through a correlation of the anti-caries and anti-microbial activities of the various agents tested.

Methods Employed: Antibiotics are tested in vivo in diets cariogenic for white rats. Caries scores are correlated with changes in the oral microflora. In addition the antibiotics are screened *in vitro* against representative strains of oral microorganisms.

Major Findings: Over the past several years a number of antibiotics have been demonstrated to be exceptionally effective in preventing experimental caries development. The most effective agents appear to belong to the "narrow spectrum" antibiotic group, i. e. penicillin, bacitracin and erythromycin. On the other hand certain antibiotic agents which have a similar spectrum of antimicrobial activity *in vitro* fail to inhibit dental caries in experimental animals, i. e. tyrothricin, subtilin, neomycin.

Significance to Dental Research: These experiments provide support to the hypothesis that the proper use of selected antibiotic agents will provide an effective means of controlling dental caries. It remains to be ascertained whether this approach is entirely feasible from the public health standpoint of safety and practicability for use in large groups of humans. The demonstration that certain antibiotic agents, which have generally similar in vitro spectrums of antimicrobial activity, differ in their ability to control caries in vivo offers an opportunity to assess the probable importance of specific oral microorganisms in the etiology of caries by a comparison of the effects of caries inhibitory and non-inhibitory drugs on pure cultures of such organisms in vitro.

Partly a result of these experiments a field study is being conducted by the Dental Health Consultant of DHEW Region III (Dr. Frank Law) on the use of a bacitracin-containing mouthwash as a possible means of caries control in school children in Culpeper and Orange, Virginia. NIDR is collaborating in this study (Fitzgerald, Jordan, Lackey, Poole, Murphy, Howell) to the extent of studying the effects on the oral microflora.

Proposed Course of Project: It is proposed that this project continue along several general lines: (a) A rat caries screening program to evaluate the potential usefulness of new antibiotic agents. Particular emphasis should be given to the search for effective agents that are not in general use in medical therapy, which are free of allergenic effects, and which do not readily induce drug-resistance microorganisms.

(b) Agents which show promise in the original screening program should be processed pharmacologically to see if they may be practicably employed in human experimentation.

(c) Carefully observed studies with clinical patients should be undertaken to work out methods of application, possibility of untoward effects, and in vivo effects on the oral microflora.

(d) Aside from these aspects of the study is the possibility of gaining clues to the microbial etiology of caries by narrowing the field of suspects through the study of the in vitro and in vivo effects of various agents with varying degrees of anti-caries activity on representative oral microorganisms. This is particularly desirable in view of the future availability of germ-free facilities for the study of pure strains of microbes in the caries problem. Other aspects that will be investigated are explorations of the possibilities, offered by the finding (Stephan) of ways to induce caries of the incisor teeth of rats, to obtain more accurate samples of the microflora at the exact site of the caries lesion.

Analysis of NIH Program Activities

Budget Data Sheet

10. NIDR-18
SERIAL NO.

11. --

12. BUDGET ACTIVITY: Research

13. IDENTIFY ANY COOPERATING UNITS: Dental Health Consultant of Region III, Dr. Frank Law, who is directing Bacitracin Mouthwash Project.

14. None

HONORS, AWARDS, AND PUBLICATIONS

15. --

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

Chapter entitled "Influence of Antibiotics on Experimental Rat Caries" in "Advances in Experimental Caries Research." AAAS Monograph. Ed. R. F. Sognnaes, Washington, D. C., 1955.

17. None.

R.P.C. - 1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

1. Dental Research
INSTITUTE

2. Oral Bacteriology
LABORATORY

3. --

4. --

5. NIDR-19
SERIAL NO.

6. The Lactobacilli of the White Rat
PROJECT TITLE

7. H. V. Jordan
PRINCIPAL INVESTIGATOR

8. R. J. Fitzgerald
OTHER INVESTIGATOR

9. PROJECT DESCRIPTION:

Objectives: The objective of these studies is to detect any changes in the types and numbers of oral lactobacilli of rats under different dietary conditions and to see if these changes correlate with dental caries in the rat. The immediate aim of the nutritional and metabolic studies is to devise better cultural methods for growth of these organisms.

Methods Employed: The oral lactobacillus populations of rats have been studied by plating samples in selective media. Rats on various dietary regimes which are known to either promote or inhibit dental caries were included. A system to distinguish the heterofermentative lactobacilli from homofermentative lactobacilli has been worked out.

Two approaches to the nutritional and metabolic studies have been employed: (a) Testing of compounds known to be active in the nutrition of the lactobacilli; (b) Attempts at the isolation and identification of growth promoting substances present in an enzymatic digest of casein.

Major Findings: The oral lactobacillus population of white rats at weanling age was found to be about 10% heterofermenter and 90% homofermenter. When these rats were placed on a diet which produces caries this percentage of heterofermenters increased to about 50%. When the rats were maintained on a noncariogenic diet the percentage of heterofermenters did not increase and it usually decreased. Generally the total numbers of lactobacilli increased if caries developed.

Nutritional and metabolic studies have centered mainly on the amino acid metabolism of the lactobacilli from rats. Aspartic acid and glutamic acid appear to be particularly important. Aspartic acid peptides have been tentatively identified during preliminary experiments on the fractionation of an enzymatic digest of casein which has a growth promoting effect.

Significance to Dental Research: In order to understand the complex interactions of the oral flora an understanding of its constituent parts is essential. Changes observed in the lactobacillus populations are an example of the type of changes which may occur and which are not apparent in a gross examination of the oral flora in dental caries studies. This type of approach could be extended to a study of the other bacterial groups which comprise the total flora of the oral environment. Any advances in understanding the metabolism of the lactobacilli have a great importance not only in the field of dental research but in the general fields of bacteriology and physiology as well. Of immediate significance is the possibility of developing better methods of culture for those lactobacilli which grow very poorly on available laboratory media and about which little is known.

Proposed Course of Project: Present studies on the changes in the lactobacillus populations of rats will be extended to observe the effect of other cariogenic and non-cariogenic diets on the oral lactobacilli and also to determine more adequate methods of sampling.

The metabolic studies will continue on essentially the same lines with emphasis on the amino acid metabolism of these organisms. Included in this area of investigation will be studies on the effect of peptides and amino acid derivatives. Results of these studies will be applied to the problem of improving presently available media for the culture of the lactobacilli, particularly the fastidious, homofermentative types.

BUDGET DATA

10. -- 11. -- 12. BUDGET ACTIVITY: Research

13. - 14. None

HONORS, AWARDS, AND PUBLICATIONS

15. - 17. None

R.P.C. - 1
December 1955 Analysis of NIH Program Activities

Project Description Sheet

1. Dental Research
INSTITUTE

2. Oral Bacteriology
LABORATORY

3. --

4. --

5. NIDR-20
SERIAL NO.

6. Variations in the Oral Microflora in Relation to Oral and Systemic Diseases.
PROJECT TITLE

7. M. D. Lackey
PRINCIPAL INVESTIGATOR

8. R. J. Fitzgerald, R. S. Lloyd, A. Howell, M. Rogosa
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION:

Objectives: This project has the following objectives: (a) To survey the aciduric oral microorganisms; (b) To establish the nature and range of variations in the oral flora as a result of various oral and systemic disease conditions before, during and after treatment; (c) To provide a diagnostic bacteriology service for the Dental Clinic in the Clinical Center; (d) To investigate bacteriological hazards resulting from the use of the high-speed water-cooled drill; (e) To study the microflora of the gingival pocket.

Methods Employed: Standard bacteriological procedures.

Current Status: At the present time, over 2,000 saliva samples have been collected in the Dental Clinic. A preliminary analysis of the data obtained from the bacteriological study of these samples has been completed, and work is underway to obtain information on oral and systemic conditions from the patients' clinical record; further analysis is dependent on the availability of these data.

The use of the high-speed water-cooled drill presents a possible bacteriological hazard to both patient and dentist. Methods of keeping the water reservoir and lines free of contamination are now being studied. Future work concerns the danger to the dentist of the aerosol, from the patient's mouth, produced by the drill.

Work on the microflora of the gingival pocket this past year has been mainly exploratory. More effective means of sampling are now being sought.

Proposed Course of Project: It is planned to continue this study.

Analysis of NIH Program Activities

Budget Data Sheet

10. NIDR-20
SERIAL NO.

11. --

12. BUDGET ACTIVITY: Research

13. - 14. None

HONORS, AWARDS, AND PUBLICATIONS

15. - 17. None.

R.P.C. - 1
December 1955 Analysis of NIH Program Activities

Project Description Sheet

1. Dental Research
INSTITUTE

2. Histology and Pathology
LABORATORY

3. --

4. --

5. NIDR-21
SERIAL NO.

6. Electron Microscopy and Electron Diffraction of Dental Tissues
PROJECT TITLE

7. David B. Scott
PRINCIPAL INVESTIGATOR

8. None
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION:

Objectives: (a) Development of new and improved techniques for preparation of specimens for electron microscopy and diffraction. (b) Determination of the ultra-fine structure of normal dental tissues, and study of the changes effected in malformation and disease. (c) Study of the physical and chemical properties of tooth enamel, with particular emphasis on reactions with fluoride compounds.

Methods Employed: Replicas and thin sections of various tissues are prepared for electron microscopy and electron diffraction. Various grinding, decalcifying, chemical treatment and physical methods are also employed.

Major Findings: (a) Technical Developments: A new positive replica method has been devised, through which studies of the crystal structure and chemical reactions of enamel can be studied. The technique, which involves preparation of shadowed collodion-carbon replicas, has as its advantages the undamaged preservation of the original specimen, mass productivity, and extreme thinness and thereby high resolution characteristics of the final film. In addition small amounts of crystalline material can be isolated on the replicas and, besides being observed microscopically as part of the preparation, can be subjected to electron diffraction and fluorescent x-ray analysis for identification. Of particular interest is the fact that areas as small as one square micron can be studied by limited area diffraction, which permits observation of the crystals within single enamel prisms and between prisms.

Methods for thin-sectioning of soft tissues are under complete revision, and at the present time work on a new technique for producing serial-type sections is nearly completed.

(b) Crystal Structure of Enamel: Observations to date by the collodion-carbon method have given indications of the sizes of enamel crystallites, and have shown that the crystals inside prisms are most likely oriented parallel to the long axis of the prisms, and that those between prisms are oriented at an acute angle to the prism axes. There have been no indications of multiple crystal orientation within prisms as suggested by other workers on the basis of X-ray diffraction data.

(c) Reactions of Enamel with Fluoride: Work in other laboratories has strongly suggested that considerable protection against etching *in vitro* by acid is imparted to enamel through treatment with stannous fluoride. This finding has been corroborated in large-scale replica studies in this laboratory, and at present experiments are in progress to determine the comparable effects of other fluoride salts, as well as to investigate why combinations of tin and fluoride seem especially effective.

Significance to Dental Research: For the first time in recent years new light is being shed on the crystal structure of enamel. The information derived through use of physical methods will help provide the data needed both for definition of the basic structure of dental tissues and for explanation of changes resulting from disease. Data from studies of reactions of fluoride compounds on enamel are especially timely in view of their widespread use in drinking water and dentifrices for the control of caries. New technical methods are of considerable value to all workers in electron microscopy, a field which is still in the stage of rapid development.

Proposed Course of Project: Continuation of all aspects of technical development, studies of crystal structure, and studies of reactions of enamel with fluoride compounds. In addition, work will be renewed on tooth development, using electron microscopy, electron diffraction and X-ray microscopy.

Analysis of NIH Program Activities

Budget Data Sheet

10. NIDR-21
SERIAL NO.

11. --

12. BUDGET ACTIVITY: Research

13. IDENTIFY ANY COOPERATING UNITS: Certain aspects of this project are being carried out in collaboration with the Section on Molecular Biophysics, Laboratory of Physical Biology, National Institute of Arthritis and Metabolic Diseases, Dr. R. W. G. Wyckoff, Chief.

14. --

HONORS, AWARDS, AND PUBLICATIONS

15. --

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

1. Scott, D. B. The electron microscopy of enamel and dentin. Annals, New York Acad. Science, 60:575 (1955).

2. Scott, D. B. and Wyckoff, R. W. G. Carbon surface replicas for electron microscopy and electron diffraction. (In press).

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

Arthur S. Flemming Award - 1955 - (Washington Jr. Chamber of Commerce).

R.P.C. - 1
December 1955 Analysis of NIH Program Activities

Project Description Sheet

1. Dental Research
INSTITUTE

2. Histology and Pathology
LABORATORY

3. --

4. --

5. NIDR-22
SERIAL NO.

6. Development and application of histochemical methods in the study of protein groups and enzyme activity of normal and abnormal tissues.
PROJECT TITLE

7. M. S. Burstone
PRINCIPAL INVESTIGATOR

8. None
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION:

Objectives: The object of this project is to develop new histochemical procedures and modifications and apply them in an attempt to demonstrate clinical differences between, and characteristics of, normal and abnormal tissues. This includes histochemical and biochemical studies of the following human tissues: (a) salivary glands, (b) normal and neoplastic tissues, (c) normal and abnormal hard and soft oral tissues.

Methods Employed: A. Histochemical methods for the visualization of protein groups. This consists of evaluating existing procedures and devising new methods and modifications. It should be pointed out that some of the older protein methods (Millon, Ninhydrine, Xanthoproteic) are essentially unsatisfactory for microscopic histochemistry.

B. Histochemical methods for demonstration of enzymes. The latter include esterases, peptidases, phosphatases, sulfatases, and glucuronidases. The use of frozen dried tissue makes it possible to utilize many poorly hydrolyzed substrates which would be unsatisfactory with conventionally fixed tissues.

C. Development of new histochemical substrates for demonstration of enzymes. The following new esterase substrates have been synthesized and an attempt will be made to use them histochemically. These substrates include naphthylmethacrylate, naphthyl cinnamate, naphthyl hydroxy cinnamate and naphthyl crotanate. Several amino acid naphthylamides are also being used.

Major Findings: A. Four histochemical procedures: the dinitrofluorobenzene, the ninhydrin-Schiff, the alloxan-Schiff, and the chloramine-Schiff methods have been found to be of use for the microscopic demonstration of certain protein groups on tissues. Good chromogenic results were observed in a variety of tissues of several species (rat, mouse, rabbit, man). (J. Histochem. & Cytochem. Vol. 3, No. 1, Jan. 1955).

B. High esterase activity of taste bud epithelium (rabbit) has been observed in frozen-dried specimens with a number of complex phenolic and naphtholic ester substrates (alpha-naphthyl butyrate, alpha-naphthyl propionate, naphthol AS acetate, alpha-naphthyl acrylate).

C. A study of acetone fixed mouse and rat salivary glands reveals serous and mucous cells to be negative (except for serous cells of the tongue), with a number of esterase substrates (naphthol AS acetate, alpha-naphthyl, alpha-naphthyl butyrate, alpha-naphthyl propionate). However, with frozen-dried tissues, discrete areas of activity are noted. Generally speaking, the active areas are associated with the more terminal portions of ducts and "special serous cells" of the submaxillary gland. Of interest is the observation that serous cells of the tongue are highly active (alpha-naphthyl acetate) while serous cells of the submaxillary gland are completely negative -- even though they appear morphologically identical. (Esterase of salivary glands. J. Histochem. & Cytochem. In press, March 1956).

D. Development of a new histochemical method for a proteolytic enzyme, and aminopeptidase. This involved the use of two chromogenic substrates, Dl-alanyl-beta-naphthylamide and L-leucyl-beta-naphthylamide. The successful demonstration of aminopeptidase with this procedure is dependent upon the use of frozen-dried material. Distribution of aminopeptidase in a variety of human tissues including salivary glands was studied. (Histochemical Demonstration of Aminopeptidase. J. Histochem. & Cytochem. In press, May 1956.

Significance to Dental Research: Since normal and abnormal characteristics of hard and soft tissues are probably characterized by basic protein and enzyme differences, newer methods and modifications for demonstrating these differences may be of value in the diagnostic visualization of changes which may not be apparent with routine morphological staining. Study of enzyme characteristics of salivary glands and other oral soft tissues may likewise elucidate basic information relating to the normal and abnormal morphological characteristics and functions of these structures.

Proposed Course of Project: Further study and evaluation of the present findings is planned. The protein methods will be employed to study human enamel,dentine and bone. The enzyme procedures which reveal certain characteristics of animal salivary glands will be utilized to study normal and abnormal human salivary glands, neoplasms, and gingiva.

BUDGET DATA

10. --

11. --

12. BUDGET ACTIVITY: Research

13. - 14. None

HONORS, AWARDS, AND PUBLICATIONS

15. --

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

    1. Burstone, M. S. An Evaluation of Histochemical Methods for Protein Groups. J. Histochem. & Cytochem., 3:32-49, Jan. 1955.
    2. Burstone, M. S. Esterase of Salivary Glands. J. Histochem. & Cytochem. In press. March 1956.
    3. Burstone, M. S. Demonstration of Proteolytic Activity in Human Neoplasms. J. Nat. Cancer Inst. In press. April 1956.
    4. Burstone, M. S. and Folk, J. E. Histochemical Demonstration of Aminopeptidase. J. Histochem. & Cytochem. In press. May 1956.

17. None

R.P.C. - 1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

1. Dental Research
INSTITUTE

2. Histology and Pathology
LABORATORY

3. --

4. --

5. NIDR-23
SERIAL NO.

6. Histochemistry of Connective Tissues
PROJECT TITLE

7. Harold M. Fullmer
PRINCIPAL INVESTIGATOR

8. None
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION:

Objectives: The objectives of this project are to use existing histochemical methods to determine chemical composition and the reactive groups of normal connective tissues, and the development of new methods to more accurately and expeditiously determine the chemical composition and reactive groups of connective tissues in normal and diseased states.

Methods Employed: Human and animal tissues are obtained from autopsy or surgical material. They are either frozen-dried or placed in various fixatives and embedded in paraffin or other material. Under controlled conditions, the sections are exposed to various reagents designed to determine the site and quantity of a reactive group or substance.

Major Findings: (a) Orcein reacts selectively with elastic tissue and connective tissues undergoing basophilic degeneration. It has been found that orcein does not behave as an acid-base dye; that it cannot be blocked by the use of any of the carbonyl, carboxyl, amine, or hydroxyl reagents; and that its reactivity is enhanced when polar groups such as carboxyl, amine, hydroxyl, and carbonyl groups are not present. Also, when orcein has combined with a substance, the compound is not broken up by acids, bases, ordinary oxidizing and reducing agents, or substances with a high dielectric constant such as urea. Acetylation of orcein renders it non-reactive with elastic tissue or tissue showing basophilic degeneration.

(b) Since several elastic tissue dyes reacted with basophilic degenerative connective tissues, the mechanism of the reaction of these dyes (Verhoeff's, Gomori's aldehyde fuschsin, and orcinol-new fuschsin) was undertaken. Blocking procedures for tissue aldehyde, hydroxyl, amine, and carbonyl groups were without effect on the subsequently carried out staining reaction. It was demonstrated that a methylation considerably more drastic than that necessary to abolish basophilia of nuclei and cytoplasm, and comparable to that required to prevent the Schiff reaction of aldehyde was required. The results suggest that these stains react with strong acid, or perhaps some form of aldehyde groups.

(c) A new stain completely selective for elastic tissue was developed, and was named orcinol-new fuschsin. This is the only stain ever developed which stains elastic tissues only. Its use in the study of arteriosclerosis and other degenerative elastic tissue diseases should be helpful.

Significance to Dental Research: Periodontitis and periodontosis are both diseases of connective tissues. It is believed that as more information is gained about the nature of normal connective tissues and their alterations in disease states, we will be in a better position to treat and prevent these diseases.

Proposed Course of Project: Next year most of the time will be devoted to the histochemical analysis of normal and diseased connective tissues.

It is hoped that sufficient equipment will be available to initiate microspectrophotometric studies of connective tissues in health and disease. This physical method of analysis will supplement the histochemical studies.

In addition, two types of very valuable material have been made available. One is some very aged mice, and the other is some lysine deficient rats. This material will be processed as indicated above to determine the changes in tissues, if any, resulting from age or lysine deficiency.

BUDGET DATA

11. --

12. BUDGET ACTIVITY: Research

13. IDENTIFY ANY COOPERATING UNITS: Certain aspects of this project are being carried out in collaboration with R. D. Lillie, Chief, Laboratory of Pathology and Histochemistry, N.I.A.M.D.

14. None

R.P.C. - 3
December 1955 Analysis of NIH Program Activities

Honors, Awards, and Publications Sheet

15. NIDR-23
SERIAL NO.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

Fullmer, Harold M., and Lillie, R. D.: A Selective Stain for Elastic Tissue (Orcinol-new fuschsin). Stain Technology. (in press)

Fullmer, Harold M., and Lillie, R. D.: Some Aspects of the Mechanism of Orcein Staining. J. Histochem. and Cytochem. (in press)

17. None

R.P.C. - 1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

1. Dental Research
INSTITUTE

2. Histology and Pathology
LABORATORY

3. --

4. --

5. NIDR-24
SERIAL NO.

6. Mineral Metabolism of Bone
PROJECT TITLE

7. William B. Savchuck
PRINCIPAL INVESTIGATOR

8. None
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION:

Objectives: To study the effects of strontium and other minerals on bone formation during development or regeneration.

Methods Employed: (a) Physico-Chemical Study: Using a colony of 160 male weanling O-M rats of an inbred strain, test groups were given strontium over periods of 90 or 180 days, following which the femora, tibiae and humeri were excised and measurements made of bone length, total volume of the intact bone, and its breaking strength under static loading.

(b) Histological Study: Upper second molars were extracted intact from rats in preliminary work to develop an experimental procedure for studying the effects of various agents on formation of actual bone in the socket. Rats were injected with Calcium-45 at various intervals after extraction. The undecalcified maxillae were embedded in plastic and sections obtained for autoradiographic study.

Major Findings: (a) On the basis of length, volume, and breaking strength, a preliminary analysis of the data on the femora shows no significant differences between the various control and strontium groups. Improved methods have been elaborated for determining the volume and breaking strength of small bones more precisely and more rapidly than previously.

(b) A rapid technique has been evolved for obtaining thin, undecalcified sections of small tooth and bone specimens by dry-grinding and polishing, thus avoiding the action of water or other solvents on the mounted section.

Significance to Dental Research: To ascertain the value of employing a clinically promising mineral supplement (strontium) in promoting postextraction or fracture healing.

Proposed Course of Project: The long bones will be analyzed spectrochemically to determine the extent of strontium deposition associated with the growth data and total mineral content.

Using the several techniques indicated above for studying various aspects of bone growth and mineralization, the influence of the so-called "rare earths" (Elements 58 through 71) on bone metabolism will be determined. This is an entirely unexplored field.

The healing of a uniform, surgically reduced fracture lesion will be followed using carrier-free Calcium-45 as a mineralization indicator.

11. --

12. BUDGET ACTIVITY: Research

13. - 15. None

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

Savchuck, William B., Effects of Strontium and Fluoride on the Repair of Unreduced Humeral Fractures in the Adult Rat. (in press)

17. None

R.P.C. - 1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

1. Dental Research
INSTITUTE

2. Epidemiology and Biometry
BRANCH

3. --

4. --

5. NIDR-25
SERIAL NO.

6. The Relationships Between Periodontal Disease and General Systemic Condition, Systemic Disease, and Aging
PROJECT TITLE

7. A. L . Russell
PRINCIPAL INVESTIGATOR

8. Norman Littleton, Carl White
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION:

Project: The relationships between periodontal disease and (a) general health, (b) systemic disease, (c) sex, (d) race, and (e) aging.

Objective: To determine whether susceptibility to periodontal disease is a manifestation of general systemic condition, or due primarily to aging.

Methods Employed: Each patient examined in the dental clinic of the Clinical Center is scored for periodontal disease. This score is then related to data obtained from the patient's medical record. The scoring method permits direct comparison with findings obtained from examination of persons not hospitalized and free of known illness. Initial analysis is proceeding on the basis of within-group comparisons.

Major Findings: Too few patient records have been processed for valid findings. On the basis of so-far inadequate evidence it appears that there may be an association between periodontal disease and rheumatic heart disease, or rheumatic fever.

Significance to Dental Research: Periodontal disease is generally considered to be the resultant of local irritating factors combined with systemic factors which interfere with tissue response and repair. Evidence as to these systemic factors is conflicting.

Proposed Course of Project: Continuation along the present lines until enough records have been processed for valid findings and logical hypotheses. This will probably require two or three years of work.

Analysis of NIH Program Activities

Budget Data Sheet

10. NIDR-25
SERIAL NO.

11. --

12. BUDGET ACTIVITY: Research

13. IDENTIFY ANY COOPERATING UNITS:

Dental Clinic Examining Staff, Clinical Center
Medical Records Section, Clinical Center

14. PARALLELS RESEARCH DONE ELSEWHERE:

None

15 - 17.

None

R.P.C. - 1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

1. Dental Research
INSTITUTE

2. Epidemiology and Biometry
BRANCH

3. --

4. --

5. NIDR-26
SERIAL NO.

6. Epidemiology of the Periodontal Diseases
PROJECT TITLE

7. A. L. Russell
PRINCIPAL INVESTIGATOR

8. R. Hayes, N. Littleton, E. M. Benjamin, C. White
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION:

Project: The epidemiology of the periodontal diseases.

Objective: To compile a descriptive epidemiology of the group of disease entities commonly called "pyorrhea."

Methods Employed: Direct examination of population groups, particularly adults and older children, followed by statistical analysis of examination data to determine relative risk under varying conditions.

Major Findings: The severity of periodontal disease (but not its inception) in an individual is related to an unknown socio-economic factor which is well described in children by the general educational level of adults in the same community, and in adults by the years of formal schooling completed by the individual. This factor explained between-county differences in 3,000 rural Indiana children aged 10-14 years examined in the summer of 1955. With this factor held constant there was no relation between severity of disease and family income, rural nature of the county, or dentists available. This same factor adequately explains differences between these children and urban children previously examined. Similar patterns were discovered in two adult groups previously examined.

No relation was found, in a group of high school children, between mean periodontal score and a proposed epidemiologic measure of traumatic occlusion, i.e. relation, in occlusion, of the maxillary and mandibular midlines.

Significance to Dental Research: There is little or no epidemiological information on periodontal disease, largely because no adequate means of scoring has previously been available. Hence the first requisite is to determine the difference between people who develop the disease and people who do not, and the factors which influence severity of the disease once it develops.

Proposed Course of Project: Field examinations of older children will be continued and extended in an effort to determine the characteristics of those children who show signs of the disease at the earlier age, and hence are presumably the more susceptible. Prevalence data from the examination of a random sample of Baltimore adults will be tabulated and analyzed. If possible another adult group will be examined in the field during the next fiscal year.

11. --

12. BUDGET ACTIVITY: Research

13. IDENTIFY ANY COOPERATING UNITS: None

14. PARALLELS RESEARCH DONE ELSEWHERE: None

15. --

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

Russell, A. L., A System of Classification and Scoring for Prevalence Surveys of Periodontal Disease. (In press)
Russell, A. L., Longitudinal Technics in the Study of Oral Disease. (Presented before American Public Health Association Annual Meeting, November 1955.) (In press)
Russell, A. L., A Study of Oral Health in Children of Prince Georges and Montgomery Counties, Maryland: Preliminary Report. (To be published in Public Health Reports)

17. None

R.P.C. - 1
December 1955 Analysis of NIH Program Activities

Project Description Sheet

1. Dental Research
INSTITUTE

2. Epidemiology and Biometry Branch
BRANCH

3. --

4. --

5. NIDR-27
SERIAL NO.

6. The Epidemiology of Dental Caries, including the Fluorine-Caries Relationship
PROJECT TITLE

7. A. L. Russell
PRINCIPAL INVESTIGATOR(S)

8. Richard L Hayes, Norman W. Littleton, Carl White
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION:

Project: Further to define the epidemiological characteristics of dental caries.

Objective: To elicit evidence as to the basic nature of this disease and the tissues it attacks so that present methods of control may be perfected and, possibly, new methods of control can be evolved.

Methods Employed: Direct examination of population groups, including groups of adults as well as children, followed by statistical analysis of examination data to determine relative risk under varying conditions.

Major Findings: Major findings during the past year include: In children aged 13-14 years newly-erupted teeth were not better protected than teeth which had been at risk for 6-8 years during the third year's use of a fluoridated water supply. Definite caries inhibition was seen in children aged 6-9 years during this period.

Significance to DENTAL Research: Data from direct observations of human populations provide a bridge whereby the findings from animal studies may be applied to human disease. Present attention is concentrated upon several areas where basic information is scanty or equivocal; for example, the analysis of the selective action of a fluoridated water used while teeth are calcifying, while young calcified enamel is maturing, and after adult enamel is present is expected to add information as to the basic mechanics and sequences of calcification and maturation of enamel.

9. PROJECT DESCRIPTION (Continued):

Proposed Course of Project: Between five and six thousand elementary and junior high school children of Montgomery and Prince Georges counties, Maryland have been examined annually for the past four years in such manner that individual children may be followed from year to year. This examination should be repeated so long as it proves profitable. Single examinations of appropriate groups will be carried out where prevalence data are incomplete or where a hypothesis is to be tested.

Analysis of NIH Program Activities

Budget Data Sheet

10. NIDR-27
SERIAL NO.

11. --

12. BUDGET ACTIVITY: Research

13. IDENTIFY ANY COOPERATING UNITS: None

14. PARALLELS RESEARCH DONE ELSEWHERE: Complements (but does not duplicate) the Grand Rapids-Muskegon fluoridation study--NIDR-29.

15. --

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

Russell, A. L.: Longitudinal Technics in the Study of Oral Disease. In Press.

Russell, A. L.: A Study of Oral Health in Children of Prince Georges and Montgomery Counties, Maryland: Preliminary Report. In Press--Public Health Reports.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955: None

R.P.C. - 1
December 1955 Analysis of NIH Program Activities

Project Description Sheet

1. Dental Research
INSTITUTE

2. Epidemiology and Biometry Branch
BRANCH

3. --

4. Bethesda & Grand Rapids Mich.
LOCATION

5. NIDR-28
SERIAL NO.

6. The Biometry of Dental Caries.
PROJECT TITLE

7. Richard L. Hayes
PRINCIPAL INVESTIGATOR

8. Carl White
OTHER INVESTIGATOR

9. PROJECT DESCRIPTION:

Project: To define certain biometric characteristics of dental caries.

Objectives: To define the role of bite-wing roentgenography in the biometry of dental caries experience and to provide a description of the eruption of the permanent teeth to define the risk factor.

Major Findings: In an investigation of school children of Grand Rapids, Michigan it was found that (1) bite-wing roentgenography confirmed the clinical finding that fluoridation caused a significant decrease in total dental caries experience, (2) on approximal surfaces, there was a large decrease in the number of cavities involving dentin but no change in the number of cavities involving only the enamel, (3) these two findings suggested that fluoride acts to inhibit the growth of cavities as well as to prevent their formation, (4) use of bite-wing X rays to supplement direct observation results in higher caries scores but also increases the variation, (5) variations in the criteria used for interpretation of X rays may cause wide variations in the reporting of dental caries experience, and (6) there is considerable variation within a single examiner even when defined criteria are used for interpretation of X rays so that caries estimates made with X rays are not necessarily more precise than those made without X rays.

In the investigation of eruption of teeth, no adequate description was found in the literature, but it appears that eruption may be described as a function of the normal probability curve and the logarithm of age. This finding needs to be confirmed more precisely.

9. PROJECT DESCRIPTION (Continued):

Significance to DENTAL Research: Roentgenography, as a supplement to direct observation, has been considered to be a method for reducing sample size, but it appears that the increased variation may lead to a requirement for larger sample sizes. Roentgenography was shown to be useful in studying lesions of different sizes. This finding will be applied in a study of first permanent molars in connection with the Grand Rapids-Muskegon fluoridation project. An adequate description of the eruption of teeth will allow one to balance the risk factor and compare groups of different ages.

Proposed Course of Project: The eruptive process will be studied with more detailed data and the risk factor further defined. Eventually it is planned that these data will be used as the basis for a description of the age distribution of dental caries experience, for the preparation of tables of expected annual increments of dental caries in connection with Dr. A. L. Russell's project on the epidemiology of dental caries and for a description of the peculiar incremental pattern found in dental caries. This peculiar pattern is also found in periodontal disease so technics evolved in this project might be applied to the study of periodontal disease.

R.P.C. -3
December 1955 Analysis of NIH Program Activities

Budget Data Sheet

10. NIDR-28
SERIAL NO.

11. --

12. BUDGET ACTIVITY: Research.

13. IDENTIFY ANY COOPERATING UNITS: None

14. PARALLELS RESEARCH DONE ELSEWHERE: The Epidemiology of Dental Caries, Including the Fluorine-Caries Relationship--NIDR-27 and The Grand Rapids-Muskegon Fluoridation Study--NIDR-29.

15. --

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955: None

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

Elected Fellow of the American Association for the Advancement of Science.

R.P.C. - 1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

1. Dental Research
INSTITUTE

2. Epidemiology and Biometry
BRANCH

3. --

4. Grand Rapids, Michigan
LOCATION

5. NIDR-29
SERIAL NO.

6. Grand Rapids-Muskegon Fluoridation Study
PROJECT TITLE

7. A. L. Russell
PRINCIPAL INVESTIGATOR

8. D. B. Scott, R. C. Likins, R. Hayes, N. Littleton, C. White
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION:

Project: Evaluation of the effectiveness of fluoridation of Grand Rapids water supply for the purpose of caries control.

Objectives: This project is designed to determine whether or not the fluoridation of a water supply will control dental caries in a community. In addition, certain aspects of the dental economics and dental manpower needs of a community are being ascertained. Factors related to caries etiology are also under consideration.

Methods Employed: This study has been in progress since 1944. During the ten years following fluoridation annual examinations were made of approximately 5,000 school children in Grand Rapids, (the study city) and about 3,000 in Muskegon (the control). Changes in dental caries prevalence were noted from year to year, and studied from the standpoint of the cause of the disease and the problems involved in treatment. Epidemiological and statistical methods were used to evaluate these factors.

At the end of this ten-year period, the ultimate result had been observed in children aged up through 11 years and Muskegon, the control city, had fluoridated its own water supply. The study is being continued on a limited scale, using the same methods, but with examinations confined to children aged 12 years or older, in whom the full inhibitory effect of fluoride is not yet evident. About 2,000 are examined annually in two Grand Rapids high schools. Examinations at Muskegon have been discontinued.

Major Findings: There has been a consistent decrease in the incidence of dental caries in Grand Rapids school children since the inauguration of the study. This change has been most pronounced in persons born since fluoridation; however a significant effect has been observed on persons whose teeth had already been formed prior to starting the study.

Results from bacteriological examinations of salivas from these children suggest that there has been a change in the oral flora. There is no evidence that this change in oral flora could have been considered prognastic of the beneficial effect observed.

Studies related to the non-dental effects of fluoridation support the belief that this level of fluoride in a water supply has no other known physiological effect.

Significance to Dental Research: This study not only proves the effectiveness and safety of water fluoridation as a carrier control procedure, it also furnishes important leads to further research into the etiology of the disease. In particular, significant findings of clinical importance are resulting from the data obtained on changes in individual and specific tooth susceptibility.

Proposed course of project: The study will be continued, on a limited scale, for about four more years, or until the full inhibitory effect has been achieved in all school-age children. Examinations will be limited to junior and senior high school students in the age range of 10 through 17 years.

It is planned that a special roentgenographic and clinical study will be made on 17-18 year-old children to determine whether the first permanent molars which were erupted at the time of the original prefluoridation examinations have received any protection against dental caries. If so, it will appear that fluoridation has a post-eruptive topical effect and may furnish protection to adults.

Analysis of NIH Program Activities

Budget Data Sheet

10. NIDR-29
SERIAL NO.

11. --

12. BUDGET ACTIVITY: Research

13. IDENTIFY ANY COOPERATING UNITS: Division of Dental Public Health, Michigan Department of Health, University of Michigan

14. PARALLELS RESEARCH DONE ELSEWHERE: The Epidemiology of Dental Caries, Including the Fluorine-Caries Relationship, NIDR-27.

R.P.C. - 4
December 1955

Analysis of NIH Program Activities

Honors, Awards, and Publications Sheet

15. NIDR-29
SERIAL NO.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

Arnold, F. A., Jr.: The Use of Fluoride Compounds for Dental Caries Control. Rept. on the Dental Health Seminar, Wellington, N. Zealand, (May 4-21, 1954), WHO, March 2, 1955.

Arnold, F. A., Jr.: Water Fluoridation for Caries Control, Tenn. State Jour., April, 1955, pp. 126-128.

Arnold, F. A., Jr.: Effect of Fluoridated Public Water Supplies on Dental Caries Prevalence. II. Summary results of the tenth year of the Grand Rapids-Muskegon Study. To be published in the Public Health Reports.

17. HONORS AND AWARDS: None

R.P.C. - 1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

1. Dental Research
INSTITUTE

2. Epidemiology and Biometry
BRANCH

3. --

4. --

5. NIDR-30
SERIAL NO.

6. The Effect of Sodium Fluoride Tablets on Dental Caries
PROJECT TITLE

7. F. A. Arnold, Jr. and F. J. McClure
PRINCIPAL INVESTIGATORS

8. C. L. White
OTHER INVESTIGATOR

9. PROJECT DESCRIPTION:

Project: The effect of sodium fluoride tablets on dental caries.

Objective: To determine if dental caries is inhibited in children who have ingested sodium fluoride, provided in the form of tablets.

Methods Employed: During the past 12-14 years, tablets containing one milligram of fluoride (F) as sodium fluoride were issued to professional personnel for administration to their children, or to children of others for whom they would assume responsibility. Depending upon the fluoride concentration of the family water supply, directions were given so that the child's fluoride ingestion (exclusive of food-borne fluoride) was adjusted to an intake equivalent to the fluoride furnished by a domestic water containing one part per million of fluoride (F). This regime was to be continued throughout the calcification period of the permanent teeth. Caries experience of these children is to be compared with the caries experience of similar children who have not received any fluoride supplement.

Major Findings: Returns and analysis are not complete.

Significance to Dental Research: It has been demonstrated that caries experience is reduced by about two-thirds after fluoridation of a community water supply. However, a considerable fraction of the national population is served by individual water supplies. If a similar caries-inhibitory effect can be demonstrated following use of sodium fluoride dispensed as tablets, the benefits now enjoyed by citizens of the community with a fluoridated water can be extended to residents of rural areas.

Proposed Course of Project: Dental examination data for children who have used the tablets will be accumulated until sufficient numbers have been reported for valid statistical analysis.

11. --

12. BUDGET ACTIVITY: Research

13 - 17. None

R.P.C. - 1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

1. Dental Research
INSTITUTE

2. Clinical Investigations
BRANCH

3. --

4. --

5. NIDR-31(C)
SERIAL NO.

6. The Physiological, Pharmacological and Toxicological Effects of Fluorides in Humans and Animals.
PROJECT TITLE

7. Nicholas C. Leone
PRINCIPAL INVESTIGATOR

8. Frank J. McClure, Isadore Zipkin, Erving F. Geever, Patricia B. Geiser
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION:

Objectives: To determine the immediate and long-range effects of fluorides by various methods of administration, on man and animals.

Methods Employed: 1. Laboratory, clinical and epidemiological studies of population groups using water with high fluoride content as compared with control groups from moderate, low and non-fluoride areas.

2. Human necropsy studies (epidemiological, chemical and histological).

3. Twenty-four hour fluoride exchange studies of normal and diseased patients (clinical).

4. Large animal studies: a. Autopsy (chemical and histological); b. Serial peripheral blood and bone marrow studies; c. Enzymes.

5. Pharmacologic studies of the intravenous and oral effects of fluorides in dogs and mice.

6. Roentgenologic epidemiological study of a population group at Framingham, Mass. (non-fluoride area).

7. Fluoride metabolic studies on hospitalized normal patients (clinical).

8. Colorado Springs (2.5 ppm F) Necropsy Study - Review of clinical history, autopsy protocols and histologic sections (980 cases).

Patient Material: (1955 Calendar Year)

1. Epidemiological studies in the field:
   Adult males and females 447

2. Clinical studies in hospital:

	Number	Average stay days
Adult males	7	10
Adult females	4	10
Children (female)	1	10

3. Human autopsies:
   from fluoride areas 22
   from non-fluoride areas 16

4. Colorado Springs Study:
   study cases 980

5. Large animal autopsies:
   fluoride 7
   normal 5

   Serial rib biopsies on live (bovines) animals:
   fluoride 10
   normal 6

   Bone marrow studies on live animals:
   fluoride 8
   normal 3

   Enzyme studies - large
   animals. 5

6. Small animals used in toxicology study:
   dogs 22
   mice 300

Major Findings: 1. In population groups using water with excessive fluorides, no detrimental physical effects were discovered except for dental mottling in those persons who had spent their tooth formative years in the area.

2. Roentgen studies of human groups show slight increased bone density and coarsened trabeculation in a small percentage of those exposed, but not to a harmful degree. In fact, beneficial adult effects have been demonstrated roentgenologically.

3. Chemical and histological analyses of human autopsy material so far have shown no detrimental effects ascribable to ingestion of fluoride in water supplies.

4. Specimens from controlled metabolic studies of fluoride exchange in normal and diseased persons are being analyzed.

5. Controlled large animal toxicity studies show that highly toxic levels of fluoride must be ingested for long periods before detectable effects can be shown.

6. Small animal studies show a wide margin of safety in the use of intravenous and oral fluoride in various amounts for experimental purposes. When interpreted in terms of human effects, there appears to be a wide margin of safety in the experimental use of fluorides intravenously or orally.

Significance to Dental Research: These data are being used to supply much needed information on the physiologic effects of fluorides on humans, under various circumstances, in response to widespread inquiries and in support of the safety of a dental fluoridation program.

Proposed Course of Project: Continuation to completion and expansion of studies listed above, where indicated.

Analysis of NIH Program Activities

Budget Data Sheet

10. NIDR-31(C)
SERIAL NO.

11. --

12. BUDGET ACTIVITY: Research

13. IDENTIFY ANY COOPERATING UNITS:

Department of Diagnostic X ray, Clinical Center, NIH and Heart Disease Epidemiological Study, NHI.

14. None

HONORS, AWARDS, AND PUBLICATIONS

15. --

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

Leone, Nicholas C., Arnold, Francis A., Jr., Zimmerman, Eugene R., Geiser, Patricia B., Lieberman, Jacob E.: A Review of the Bartlett-Cameron Survey - A Ten-Year Fluoride Study. J.A.D.A., 50: 277, March 1955.

Zimmerman, Eugene R., Arnold, Francis A., Jr., Leone, Nicholas C.: The Oral Aspects of Excessive Fluoride in a Water Supply - A Ten-Year Study. J.A.D.A., 50: 272, March 1955.

Leone, Nicholas C.: Fluorides in Everyday Life. Texas State Journal of Medicine, 51: 7, 457, July 1955.

Leone, Nicholas C., Stevenson, Clyde A., Hilbish, Theodore F., Sosman, Merrill C.: A Roentgenologic Study of a Human Population Exposed to High-Fluoride Domestic Water - A Ten-Year Study. American Journal of Roentgenology, 74: 5, 874-885, November 1955.

Leone, Nicholas C., Moran, Neil, Geever, Erving F., with the technical assistance of Butler, William M., Jr., Geiser, Patricia B., Watts, James S.: Acute Toxicity Studies of Sodium Fluoride in Animals. Public Health Reports (In press).

17. None

R.P.C. - 1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

1. Dental Research
INSTITUTE

2. Clinical Investigations
BRANCH

3. --

4. --

5. NIDR-32(C)
SERIAL NO.

6. The Relationship of Certain Systemic Conditions and Oral Disease
PROJECT TITLE

7. N. C. Leone, R. J. Fitzgerald, J. Lynch and E. M. Benjamin
PRINCIPAL INVESTIGATORS

8. P. B. Geiser
OTHER INVESTIGATOR

9. PROJECT DESCRIPTION: Three separate studies:

(a) Calcium Study---A study of the measurement and clinical significance of protein bound and unbound (ionizable) calcium in blood.

(b) Dysmenorrhea Study--Treatment of dysmenorrhea by the use of Chlorprophenpyridamine Maleate, 12 mgm. (antihistaminic) sustained release capsules and the evaluation of attendant gingival change.

(c) Periodontal Clinical Study--Clinical study of the etiology of periodontal disease.

Objectives: Respectively: (a) To develop simplified and reliable methods of measuring bound and unbound calcium in blood serum and to apply the findings in terms of various clinical circumstances.

(b) To evaluate the effect of this medication on the discomfort associated with menses and tissue response of the mucous membranes of the body to this medication, with particular reference to the gingiva and oral mucosa as a whole.

(c) To obtain information on general systemic conditions which may be factors in the etiology of periodontal disease.

Methods Employed: (a) Blood from patients and large animals with normal and abnormal blood as well as exposure to known levels of fluoride. A new type of apparatus has been devised for the separation of bound and physiologic calcium.

(b) Adult females with long history of severe dysmenorrhea. Medical history, physical examination including pelvic examination made. Also, vaginal smear for cancer and culture for trichomonas and monilia made. Gingival examination and evaluation with dental prophylaxis at start of study and subsequent examinations made at intervals during patients' menstrual cycle. Daily patient records of symptoms, medications, etc. throughtout the study. Each patient under study for an initial period of six months, three months of which they are given medication and three months placebos. A system of randomization, set up by the study statistician with arrangements for the distribution of the medication through the Clinical Center Pharmacy. Members of the study team have no knowledge as to when the respective participant is receiving placebos or antihistaminic.

(c) Complete physical and dental evaluation of patients including extensive family history, past and current medical history, review of systems, laboratory studies of blood, urine and other body fluids and X-ray bone and dental studies. All patients to be hospitalized and studied on identical basis.

Patient Material: (1955 calendar year)

		Number	Days in Hospital
1. Calcium Study:	In-patient admissions	7	1
	Out-patients (7 visits)	7	
	Animals	9	

There has also been obtained 2½ liters of fresh serum from a normal adult bovine as a control pool for initial experimental procedures.

2. Dysmenorrhea Study: Female employees from the National Institutes of Health

Number of Patients (1955)	5
Number of Visits	10

3. Periodontal Clinical Study: A statistically satisfactory number of male and female adults with primary and maximal periodontal disease as compared with "normal" patients.

Hospital beds for all NIDR studies were made available during the last month and one-half of 1955 and, therefore, beds were utilized for a back-log of oral surgical and other NIDR projects. Extensive use of bed space for the study of periodontal disease has been initiated with an anticipated total of 50 patients, averaging ten hospital days each, for this study alone during the year 1956.

Major Findings: All three studies are in preliminary stages.

Significance to Dental Research: (a) Important in the evaluation of bone and tooth formation, growth and development in terms of age, exposure to fluorides, and other substances as well as in the presence or absence of oral infections, particularly periodontal disease.

(b) To measure the response of gingival tissues as well as other body tissues to the medication given, as well as careful observation of gingival as well as other oral tissue changes during menses. To determine if oral changes occur in dysmenorrhea.

(c) Of extensive importance in combating the number one dental problem in man. Correlating systemic with dental findings in an attempt to establish etiologic factors of periodontal disease, to date unknown or unidentified.

Proposed Course of Projects: Continuation and expansion of all activities.

BUDGET DATA

10. --

11. --

12. BUDGET ACTIVITY: Research

13. IDENTIFY ANY COOPERATING UNITS: Jacob Lieberman, Office of the Director, and Mrs. Frances Wolford, Employee Health Service, NIH

14. --

HONORS, AWARDS, AND PUBLICATIONS

15. - 17. None

R.P.C. - 1
December 1955 Analysis of NIH Program Activities

Project Description Sheet

1. Dental Research
INSTITUTE

2. Clinical Investigations
BRANCH

3. --

4. --

5. NIDR-33(C)
SERIAL NO.

6. Clinical Studies of the Effect of Specific Intravenous Therapy on Wound Healing in the Oral Cavity and on Cases of Intractable Gingivo-Stomatitis.
PROJECT TITLE

7. R. S. Lloyd, N. C. Leone and E. J. Driscoll
PRINCIPAL INVESTIGATORS

8. --

9. PROJECT DESCRIPTION:

Objectives: There is reason to believe, based on previous research, that certain vitamin preparations (notably B, B complex and C), when given intravenously, may favorably alter the body tissue response to injury, infection and other extraneous insults.

The purpose of the present project is to test objectively the hypothesis of beneficial effect of these intravenous medications, using the tissues of the oral cavity as a yardstick in the measurements.

Methods Employed: All patients are given a thorough physical examination, in order to properly disclose and evaluate underlying physical disorders which might confuse or alter the final results. A two-phase approach is being made to the problem of evaluation:

1. Bilateral oral surgical conditions of a similar nature are being operated upon. The surgery is performed on one side without the co-administration of the intravenous medication and serves as a control. The opposite side is the experimental side and the surgery is performed along with the intravenous medication regimen.

The degree of swelling, pain, bleeding and trismus serve as objective criteria in the evaluation of the effect of the therapy. This phase is intended to measure the tissue healing potential of the drugs.

2. The second phase is designed to measure the healing as well as the anti-infection and anti-inflammatory properties of the agents. For this project, patients with gingivo-stomatitis have been chosen. Desquamative and desquamative-like cases of gingivo-stomatitis are being utilized as the yardstick of the efficiency

of the remedy in this phase. All of the patients are given an oral prophylaxis and the mouth is put in as good order as possible. Then half of the patients are treated with the intravenous preparation and the other half are given placebos and are used as controls. Again suitable objective criteria have been set up for the measurement of the results.

Patient Material: (1955 calendar year)

Bilateral Oral Surgical Procedures:

DENTAL	Controls	20
	Experimental	25

		No.	Days in Hospital
Inpatient admissions:	Adult males	1	14
MEDICAL	Adult females	3	14
	Children female	1	14

Outpatient:	Number of patients	31
	Number of visits	180

Major Findings: 1. (a) (In multiple extractions). The response has been better than in the case of impacted teeth. The reason for this is not clear. (b) There appears to be less ecchymosis and swelling, but the pain is not materially altered.

2. There were not enough cases in the stomatitis series for any evaluation.

Significance to Dental Research: The rate at which tissue heals, as well as the conditions which surround healing are of paramount importance in oral surgery and oral medicine.

The possibility exists that certain routine post-operative sequalae such as pain, edema, hemorrhage and trismus may be favorably altered by therapy.

If tissue healing can be assisted and resistance can be increased, then many of the intractable cases of stomatitis may be improved by this approach.

Proposed Course of Project: Continuation and expansion of activities to include more oral surgery and stomatitis cases for study and evaluation of the drugs.

Analysis of NIH Program Activities

Budget Data Sheet

10. NIDR-33(C)
SERIAL NO.

11. --

12. BUDGET ACTIVITY: Research

13. IDENTIFY ANY COOPERATING UNITS: Jacob Lieberman, Office of the Director, NIH

14. None

HONORS, AWARDS, AND PUBLICATIONS

15. - 17. None.

Analysis of NIH Program Activities

Project Description Sheet

1. Dental Research
INSTITUTE

2. Clinical Investigations
BRANCH

3. --

4. --

5. NIDR-34(C)
SERIAL NO.

6. General anesthesia (dental) baseline data
PROJECT TITLE

7. F. J. Driscoll
PRINCIPAL INVESTIGATOR

8. R. S. Lloyd
OTHER INVESTIGATOR

9. PROJECT DESCRIPTION:

Objectives: Dental general anesthesia is different from ordinary hospital anesthesias in several important respects: (a) The patients are ambulatory and not usually sedated; (b) the operations are performed in as light a plane as possible; (c) the operations are performed in or about the airway.

Because of these major differences, the background of knowledge in general hospital anesthesia is not for the most part complete, accurate or comparable.

There is a great need in dental anesthesia for a background of fundamental data concerning the alterations in the physiological mechanisms. The body functions may be altered within certain limits, beyond these limits exist great hazards. One of the purposes of the study is to define these limits. The accumulated data will also be used as a baseline of comparison for the many new drugs which are being proposed for use in oral surgery.

Methods Employed: Various oral surgical procedures are being performed under general anesthesia. (Sodium pentothal, nitrous oxide, Vinethene, Trilene combinations).

Physiologic status and alterations being measured are: (a) The effects of the drugs on blood pressure and pulse; (b) Cyanosis, syncope and other circulatory changes; (c) The response of the heart, arrythmias, etc.; (d) Apnea, hypernea, tidal volume and other respiratory phenomena.

The ordinary instruments employed in physical diagnosis such as sphygomanometer and stethoscope and routine methods are at present being employed. As the study progresses, special instruments like the electrocardiograph, the sphgmotonograph, and possibly the oximeter may be employed.

Important technical data such as amount of drug used in units of time, length of operation and duration of sleep will be recorded.

Pertinent patient data, such as age, race, sex, emotional status, in relation to the conduct of anesthesia are being recorded, as well as post anesthetic sequalae, like headache, nausea and vomiting, depression and hiccoughs.

Patient Material: (1955 Calendar Year)

This project was not carried out in 1955: (a) Because of scarcity of outpatients (good risk) needing oral surgery. Several new sources of patients are being investigated and they look promising; (b) Turnover of personnel trained to work under general anesthesia; (c) Lack of Medical Board approval of the project. An anesthesiologist or trained nurse anesthetist is being sought to work with the oral surgeon, who is trained in general anesthesiology. With such a trained team, Medical Board approval seems very probable.

Major Findings: See paragraph above.

Significance to Dental Research: The search is constantly being made in dentistry for safer general anesthetics and without such baseline material the search has no foundation. The possibility also exists for improvement and refinement of present methods as well as laying a sound foundation for further anesthesiology research.

Proposed Course of Project: For the most part the initial study will be in gathering these data on various types of good risk patients, particularly outpatients. Later, after sufficient material has been accumulated, the newer drugs will be evaluated.

BUDGET DATA

10. --   11. --

12. BUDGET ACTIVITY: Research   13. - 14. None

HONORS, AWARDS, AND PUBLICATIONS

15. - 17. None

NOTE: Dr. Lloyd is Chief of the Dental Department in the Clinical Center.

R.P.C. - 1
December 1955 Analysis of NIH Program Activity

Project Description Sheet

1. Dental Research
INSTITUTE

2. Clinical Investigations
BRANCH

3. --

4. --

5. NIDR-35(C)
SERIAL NO.

6. Study of Cysts and Radioluscent Areas
PROJECT TITLE

7. Edward J. Driscoll
PRINCIPAL INVESTIGATOR

8. M. S. Burstone and Harold R. Stanley, Jr.
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION:

Objectives: The project is designed: (a) To study previously untried or little tried diagnostic methods such as aspiration and punch biopsies.

(b) To see if there are any histochemical similarities or consistent differences between granulomas and cysts.

(c) To study the surgical treatment of these areas by comparative methods, i.e. enucleation, marsupialization, etc.

Methods Employed: (a) Patients are given a thorough physical examination to disclose any underlying or complicating disease.

(b) The lesions are treated surgically and the material which is removed, whether granulomatous or cystic, is sent to the laboratory for histochemical and other histopathological analysis.

(c) The various diagnostic methods are employed and recorded for later analysis.

(d) The lesions are treated by various surgical means, such as open and closed method, gelfoam penicillin space filling, etc., and the data is accurately compiled for later comparative study.

Patient Material:

Outpatient:	Number of patients		12
	Number of visits		96
	Large cysts:	radicular	3
		follicular	3
	Granulomas:		6

Major Findings: Gelfoam - penicillin has been described as an excellent space filler for the large cystic areas. This has been corroborated in that postoperative periods are measured in weeks rather than months.

Significance to Dental Research: (a) Very little is known about the etiology of cysts. It is hoped by studying the lesions with modern laboratory methods that some light might be thrown on their etiology.

(b) Radicular cysts are thought to be a later stage of degeneration of periapical granulomas. Phases of this hypothesis are being studied. If radicular cysts are not preceded by periapical granulomas, this will lend weight to the anti-curretment movement in oral surgery.

Proposed Course of Project: Continuation to include as many cases of cysts and granulomas as possible.

BUDGET DATA

10. --

11. --

12. BUDGET ACTIVITY: Research

13. - 14. None

HONORS, AWARDS, AND PUBLICATIONS

15. - 17. None

R.P.C. - 1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

1. Dental Research
INSTITUTE

2. Clinical Investigations
BRANCH

3. --

4. --

5. NIDR-36(C)
SERIAL NO.

6. Genetic Study in Prince Georges and Charles County, Maryland and the State of Michigan.
PROJECT TITLE

7. Carl J. Witkop
PRINCIPAL INVESTIGATOR

8. Hazel Dyson, Shirley Butters
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION:

Objectives: To observe the results of genetic and environmental factors at work in a group of humans who have inmarried for several centuries. To characterize and evaluate the effect of hereditary and environmental factors on mental illness, dental disease, cardiovascular disease, metabolic disease and neurological disease as it occurs in this group. To clarify the diagnostic criteria for hereditary defects in enamel and dentin. To determine the prevalence, transmission, clinical description of these defects and the cellular and chemical level where these genetic traits have their primary action.

Methods Employed: A pedigree and census of the group is being made. Each individual is interviewed by nurses and a medical questionaire is answered. Each individual is given a medical and dental examination. Selected patients are brought to the Clinical Center for detailed tests and examinations. Samples of blood, urine, saliva, and genetic tests are made on each individual. Biochemical and bacteriological examinations are made on these samples. Linkage tests are made. All data is coded for IBM punch card analysis. Histochemical methods are being worked out to determine the nature of the biochemical defect in the tooth defects. The same techniques are being applied to the Michigan portion of the study. The use of genetic strains of mice with similar defects in bone and tooth development is being studied.

Major Findings: 1. A racial isolate comprising 5000 related individuals, many having morphological and biochemical hereditary defects has been characterized.

2. The defects discovered in these people which may have a genetic factor in their manifestation are: dentinogenesis imperfecta, polycystic kidneys, lop ears, albinism, cystic fibrosis of the pancrease, hypertension, Albers-Schönberg's osteopetrosis, sickle-cell anemia, schizophrenia, third nerve paralysis, asthma, epilipsy, diabetes, deaf-mutism, blue sclera, mental deficiency and glaucoma.

3. Some 26 tri-racial isolates exist in this country.

4. One defect, dentinogenesis imperfecta, has been shown to be a single entity and that prior separation of this condition into several diseases simply represents the variations in the manifestation of the genetic defect. This condition occurs once in 7000 individuals.

5. Four and probably five genetic defects in enamel formation were discovered in Michigan. These separate clinical and genetic entities were previously thought to represent two variants of amelogenesis imperfecta. These conditions occur once in 14,000 individuals.

6. An unusual association of conditions occurring in families both in Michigan and Maryland was found. These conditions, which may have some common biochemical link are dentinogenesis imperfecta, hypertension, albinism and schizophrenia.

Significance to Dental Research: 1. Over 20,000 individuals living in the U. S. are affected with hereditary disturbances in enamel and dentin formation. These conditions have been poorly described. Little is known about their transmission, prevalence, treatment or prevention. In many instances these conditions are confused with fluorosis.

2. The study of large in-married groups of individuals has not been done from the medical and genetic viewpoint. Here are possible metabolic links between medical and dental conditions.

Proposed Course of Project: To combine these studies in epidemiology, genetics, chemistry, etc., into one report to be completed in five years. To integrate various clinical, dental and medical investigations on these patients and others to form the nucleus for a Human Hereditary Clinic at NIH.

Analysis of NIH Program Activities

Budget Data Sheet

10. NIDR-36(C)
SERIAL NO.

11. --

12. BUDGET ACTIVITY: Research

13. IDENTIFY ANY COOPERATING UNITS:

1. National Institute of Mental Health
2. National Institute of Neurological Diseases and Blindness
3. National Institute of Arthritis and Metabolic Diseases
4. National Heart Institute
5. National Cancer Institute
6. National Microbiological Institute
7. Division of Biologic Standards
8. Research Grants
9. Howard University College of Dentistry
10. Catholic University Department of Sociology

14. --

HONORS, AWARDS, AND PUBLICATIONS

15. --

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

Hursey, R. J., Witkop, C. J., Miklashek, D. and Sackett, L. M.: Dentinogenesis Imperfecta in a Racial Isolate with Multiple Hereditary Defects. Oral Surg., Oral Med., and Oral Path. (In press).

17. None

R.P.C. - 1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

1. Dental Research
INSTITUTE

2. Clinical Investigations
BRANCH

3. --

4. --

5. NIDR-37(C)
SERIAL NO.

6. A Clinical Trial to Compare Treatment of Periodontitis by Curettage and Gingivectomy
PROJECT TITLE

7. E. M. Benjamin
PRINCIPAL INVESTIGATOR

8. None
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION:

Objectives: Comparative evaluation of two methods of periodontal treatment.

Methods Employed: Patients having a diagnosis of periodontitis simplex are treated in alternate quadrants of the mouth by curettage and gingivectomy. Evaluations are done at one and six months using pocket depth, length of clinical crown, and radiographic interpretation of bite wing X rays as criteria.

Patient Material:

Out-patients	12
Visits	84

Major Findings: 1. Gingivectomy reduces pocket depth about twice as much as curettage one month post treatment.

2. Both treatments elongated the clinical crowns of the teeth. Gingivectomy elongated the crowns more than curettage in the majority of cases.

3. No changes were seen in the bone from interpretation of bite wing X rays.

Significance to Dental Research: Aids in treatment choice by demonstrating exactly what response may be expected following these two types of treatment in normal patients.

A method was developed by which two different treatments can be compared necessitating the use of relatively few patients.

Proposed Course of Project: Comparisons of other types of periodontal treatment are planned. The response of patients with known systemic disease is to be studied.

BUDGET DATA

10. --

11. --

12. BUDGET ACTIVITY: Research

13. - 14. None

HONORS, AWARDS, AND PUBLICATIONS

15. --

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

Benjamin, E. M. The Quantitative Comparison of Curettage and Gingivectomy in the Treatment of Periodontitis Simplex. (In press).

17. None

R.P.C. - 1
December 1955 Analysis of NIH Program Activities

Project Description Sheet

1. Dental Research
INSTITUTE

2. Clinical Investigations
BRANCH

3. --

4. --

5. NIDR-38(C)
SERIAL NO.

6. A Study of the Reattachment of Supporting Structure to a Tooth Following the Creation of a Periodontal Pocket by Surgical Means.
PROJECT TITLE

7. E. M. Benjamin
PRINCIPAL INVESTIGATOR

8. Harold R. Stanley
OTHER INVESTIGATOR

9. PROJECT DESCRIPTION:

Objectives: 1. To find whether or not reattachment of supporting structure occurs in pockets so created.

2. Effects of various periodontal treatments on histologic components of tooth and supporting structure in surgically created pockets.

Methods Employed: Pockets are created in patients having essentially periodontally sound teeth whose teeth are to be extracted for therapeutic reasons. Bands of inert metal with a metal tongue reaching to the bottom of the pocket are cemented on the teeth for two weeks. Cultures are taken. Periodontal treatment of some teeth is done. Others are left alone. Sections are removed for histological study at designated intervals.

Patient Material:

Out-patients	2
Visits	42

Major Findings: Healing of the pockets so created in both treated and untreated teeth was observed. Treatment: curettage and polish.

Significance to Dental Research: Shows histological response of tissues following surgical creation of a pocket with and without treatment.

Proposed Course of Project: More patients are to be done and other types of periodontal treatment tried. Tissues will be removed at different intervals.

BUDGET DATA

10. --

11. --

12. BUDGET ACTIVITY: Research

13. - 14. None

HONORS, AWARDS, AND PUBLICATIONS

15. - 17. None

R.P.C. - 1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

1. Dental Research
INSTITUTE

2. Clinical Investigations
BRANCH

3. --

4. --

5. NIDR-39(C)
SERIAL NO.

6. Histopathologic Study of Periodontal Disease.
PROJECT TITLE

7. Harold R. Stanley, Jr.
PRINCIPAL INVESTIGATOR

8. None
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION:

Objectives: 1. To assess the constancy of specific distances within the anatomic components of the periodontal tissues in serial sections of periodontal specimens and how these distances fluctuate in relation to local pathologic factors.

2. To determine how many sections per millimeter of tissue need to be studied in order to observe all obvious structural variations that might occur.

3. To evaluate the Aisenberg hypothesis of epithelial attachment migration.

4. To investigate the possibility of the lateral spread of calculous plaques beneath intact epithelial attachments.

Methods Employed: Serial sectioning of blocks of human teeth with intact periodontal tissues. Six specific distances are measured at a magnification of x100. A stage micrometer is employed. Differences in these distances reflect changes in the anatomic components as successive sections of blocks are studied and these differences are statistically analyzed in relation to local pathologic factors. Camera lucida drawings and photography will be used to establish in three dimensions the course of the epithelial attachments and the lateral extensions of calculous plaques.

Major Findings: Data indicates that essentially the same results are obtained by the measurement of every 20th section as every section or every fifth section. However, the procedure requires that the number of sections examined be sufficiently large to guarantee a valid estimate of the distribution, that is, provides a representative sampling. In the material collected, distribution appears adequately described by 8 to 10 sections per millimeter of tissue. The most constant distances appear to be from the bottom of the calculous plaque to the alveolar crest, from the gingival crest to the alveolar crest and from the bottom of the calculous plaque to the bottom of the epithelial attachment. These appear to be reliable aids for the morphologic comparison of periodontal lesions of different specimens.

Certain types of individuals appear to be capable of epithelial attachment migration as described by Eisenberg but the majority of individuals do not.

Some periodontal specimens reveal the apparent undermining of epithelial attachments by the lateral growth of calculous plaques. The epithelial attachment is attached in its upper portion but detached in its lower segment.

Proposed Course of Project: 1. Continue to obtain and section as many specimens of human jaws as possible in order to accumulate an adequate collection of periodontal tissues.

2. Examine carefully the clinical appearance of the periodontal tissues in respect to the presence or absence of calculus and the depths of pockets before fixation. Photographs will be taken at this time.

3. Make suitable markings on the teeth relative to the position of the soft tissues in order to establish the degree of shrinkage of the soft tissues induced by fixation.

Significance to Dental Research: In order to treat periodontal disease effectively, more precise histo-morphologic criteria need to be established in relation to the clinical appearance of the disease.

Analysis of NIH Program Activities

Budget Data Sheet

10. NIDR-39(C)
SERIAL NO.

11. --

12. BUDGET ACTIVITY: Research

13. - 14. None

HONORS, AWARDS, AND PUBLICATIONS

15. --

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

    1. Stanley, H. R., Jr. The Cyclic Phenomenon of Periodontitis, Oral Surg., Oral Med., and Oral Path. 8: 598-610, 1955.

    2. Stanley, H. R., Jr. The Pathology of Periodontal Disease, from the text The Management of Oral Disease, by Col. J. L. Bernier.

17. None

Analysis of NIH Program Activities

Project Description Sheet

1. Dental Research
INSTITUTE

2. Clinical Investigations
BRANCH

3. --

4. --

5. NIDR-40(C)
SERIAL NO.

6. Sodium Dilantin Toxicity in Swiss Mice
PROJECT TITLE

7. H. R. Stanley, Jr.
PRINCIPAL INVESTIGATOR

8. None
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION:

Objectives: To determine the maximum tolerated dosage. To develop animals resistant to the drug. To possibly interfere with the growth, histo-differentiation and morphodifferentiation of the dental organ.

Methods Employed: Daily intraperitoneal injections of subconvulsive and convulsive doses of sodium dilantin in adult males, adult females, pregnant females and infant mice. Daily records are kept of weight variations, clinical lesions and animal behavior. Complete autopsies are performed on all animals.

Major Findings: The convulsive dose induces convulsions only the first few days. Thereafter a convulsion is seen only occasionally. Adult males cannot tolerate the dose nor can non-pregnant females. Primiparae cannot tolerate the dose but females Gravida II or more can be successfully brought through to delivery. The litters will be small and retarded but will live. Although mucous plugs are found most of the primiparae will not deliver litters.

With a sub-convulsive dose more litters can be obtained with primiparae who are often incapable of nursing and therefore eat the infants. Many infants will develop bloated abdomens due to the swallowing of air in attempted nursing. No disturbance of odontogenesis has been observed as yet.

Significance to Dental Research: Thousands of epileptics undergo a gingivectomy, often repeatedly due to sodium-dilantin therapy. A controversy exists as to whether the gingival hypertrophy is due to a preceding gingivitis or to the dilantin itself. A controlled disturbance in odontogenesis in the new born without gingivitis would indicate that the odontogenic tissues are specifically disturbed by the sodium dilantin. If a similar lesions could be manifested in animals, which has never been done, perhaps an adjustment could be made in human dilantin therapy, either by adding or eliminating some factor that would not hinder the efficiency of controlling epilepsy but inhibit gingival hypertrophy.

Proposed Course of Project: At this time a recognized resistant animal has not been established. Possibly the surviving litters of females receiving convulsive doses during gestation will provide such animals but it is too early in the project to tell at this time.

It is planned to study the adrenals of these animals as well as other systems to detect symptoms of Selye's adaptation syndrome. In many animals, it is obvious that an "exhaustion" stage has been reached at the time of the animal's death.

If no disturbance of the gingival tissues is found under these conditions, a periodontal disease-inducing-diet will be substituted for the normal diet now employed.

BUDGET DATA

10. --

11. --

12. BUDGET ACTIVITY: Research

13 - 14. None

HONORS, AWARDS, AND PUBLICATIONS

15 - 17. None

R.P.C. - 1
December 1955 Analysis of NIH Program Activities

Project Description Sheet

1. Dental Research
INSTITUTE

2. Clinical Investigations
BRANCH

3. --

4. --

5. NIDR-41(C)
SERIAL NO.

6. Pulpal Response to High Speed Cutting and Grinding of Teeth.
PROJECT TITLE

7. H. Swerdlow and H. R. Stanley
PRINCIPAL INVESTIGATOR(S)

8. None
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION:

Objectives: This study is designed to evaluate the effects on the dental pulp following standard operative procedures performed at high r.p.m. with and without a coolant and various types of rotary instruments.

Methods Employed: Patients of either sex and any age over six (6) are used. They must have non-infected, caries free teeth to be extracted for reason such as:

(1) Orthodontic therapy.
(2) Hopeless periodontal prognosis.
(3) Unusable malpositioned teeth, i.e. prosthesis.

Following extraction these teeth are histologically studied by Dr. H. R. Stanley, Oral Pathologist.

Patient Material: (1955 calendar year). 13 patients with 67 teeth prepared for histologic study.

Major Findings: Discreet lesions are found in the pulp for those teeth prepared with and without a coolant. Definite histologic stages of breakdown and healing are being developed.

Significance to Dental Research: The tendency today in operative procedures is for higher rotary speeds with little regard to pulp damage. Clinical information concerning these pulpal reactions is greatly needed in order to determine the safest and most efficient methods for cavity preparation.

Proposed Course of Project: To continue investigations of the pulpal responses to higher rotary speeds, varying rotary instruments and other standard operative procedures.

BUDGET DATA

10. --

11. --

12. BUDGET ACTIVITY: Research

13 - 14. none.

HONORS, AWARDS, AND PUBLICATIONS

15 - 17. none.

NOTE: Dr. Swerdlow is under the employ of the Clinical Center, Professional Services Department.

R.P.C. - 1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

1. Dental Research
INSTITUTE

2. Clinical Investigations
BRANCH

3. --

4. --

5. NIDR-42(C)
SERIAL NO.

6. Malocclusion as Related to Traumatic Occlusion.
PROJECT TITLE

7. H. Swerdlow and A. W. Mann
PRINCIPAL INVESTIGATORS

8. None
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION:

Objectives: To evaluate the effects of balancing occlusion by selective grinding of natural dentition on the periodontal tissues (the effects on facial muscle tonus and the temporo-mandibular joint will also be studied).

Methods Employed: Adult patients with approximately 28 teeth in occlusion are selected. A demonstrable though slight malocclusion and some gross evidence of periodontal disease must be present. The existing occlusion will be recorded on an anatomical articulator and all necessary pre-treatment records are taken i. e. mandibular movements, photographs, roentgenographs, etc. After functional analysis, the occlusion will be corrected and the clinical course of the disease charted.

Patient Material: (1955)

Outpatients:	Number of patients	21
	Number of visits	105

Major Findings: The preliminary work done thus far has helped to delineate the methods of approach. Special tools stimulated by this study are being developed, such as:

1. A clinical camera in order to accurately measure change in gingival color and contour.

2. A method for recording the sounds of occluding teeth on tape, demonstrating premature occlusal contacts versus balanced occlusion.

Significance to Dental Research: Periodontal disease is the most important cause of the loss of teeth at the present time. It is generally accepted that malocclusions and malfunctions of the teeth are important local causative factors in periodontal disease. It is usually noted that the occlusal pattern is unbalanced and not in harmony with the temporo-mandibular joint when periodontal disease is present. This affects not only the tissues adjacent to the teeth but also the facial muscles, ligaments and other allied structures.

Proposed Course of Project: It is hoped that tools being developed will aid in the measurement of the changes that occur in the teeth and surrounding structures. Accurate findings in this study will help to determine the value of this type of therapy in periodontal disease.

BUDGET DATA

10. --	11. --
12. BUDGET ACTIVITY: Research	13. - 14. None

HONORS, AWARDS, AND PUBLICATIONS

15. - 17. None

NOTE: Dr. A. W. Mann is a consultant to NIDR.
Dr. H. Swerdlow is under the employ of the Clinical Center, Professional Services Department.

R.P.C. - 1
December 1955 Analysis of NIH Program Activities

Project Description Sheet

1. Dental Research
INSTITUTE

2. Clinical Investigations
BRANCH

3. --

4. --

5. NIDR-43(C)
SERIAL NO.

6. Growth and Development of the Head in Normal and Abnormal States in Man.
PROJECT TITLE

7. S. Pruzansky and J. D. Subtelny
PRINCIPAL INVESTIGATORS

8. R. S. Lloyd
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION:

Objectives: The object of this investigation is to (a) extend the usefulness of cephalometric roentgenography as a research instrument in analyzing the growth of the head in man and understanding the nature of deviations from the normal range of variation.

(b) To analyze the synergistic contribution of the muscles of mastication to the movements of the mandible under varying conditions affecting the mobility of the lower jaw and the posture of the head.

(c) To investigate the mechanisms by which favorable or unfavorable results are achieved in orthodontic therapy.

Methods Employed: The methods employed for documentation and analysis of clinical observations include cephalometric roentgenography, electromyography and articulated casts of the upper and lower dentition.

The standardized Broadbent-Bolton cephalometer is utilized for studies on older children and adults. Films are obtained in occlusion rest, wide open and during the sustained phonation of vowels. The effect of various prosthetic appliances on the posture of the mandible, hyoid bone, tongue and soft palate is analyzed from these films. In addition, a modification of the Broadbent-Bolton cephalometer suitable for studies on infants is employed. The technic requires that the infants be sedated to insure immobility during the exposure.

The electromyographic equipment consists of Offner Electronics Company amplifiers and crystograph recorders utilizing four channels for simultaneous recording. The output of any one of the preamplifiers may be switched to the input of an oscilloscope or loudspeaker. Surface and needle electrodes are employed to pick up the electrical activity of the temporal and masseter muscles. The synergistic activity of these muscles is studied at rest, in mandibular movements, and in a variety of pathologic conditions affecting the neurophysiological control of the posture and motion of the lower jaw.

Articulated orthodontic casts of the upper and lower jaws serve as an invaluable adjunct to the other records in that they afford detailed information concerning important variables in occlusal relations.

Patient Material: The following figures are incomplete for 1955 since both investigators left NIDR during the year.

Outpatients:	Number of patients	95 - on 7 separate studies
	Number of visits	500

Major Findings: 1. Various electromyographic patterns obtained during chewing were found to be characteristic of certain types of malocclusion. The contribution of the temporal and masseter muscles to the posture of the mandible has been analyzed.

2. The mechanisms by which rapid changes are achieved through orthodontic expansion in bilateral cleft palate has been described.

3. A technic for the fabrication and design of speech appliances for young children with unoperated cleft palate was developed.

Significance to Dental Research: To obtain a better understanding of the normal processes of the growth of the head in man, it is necessary to analyze a variety of congenital and acquired anomalies affecting the growth and development of the head. Such information may aid in the development of better diagnostic methods and serve to establish a scientific basis for therapy.

The synergistic contribution of the muscles of mastication to the movements of the mandible and to its postural control is essential for a complete understanding of the masticatory mechanism. The application of neurophysiological methods to the study of dental problems offers virgin territory for useful exploration.

Proposed Course of Project: This study has been temporarily suspended and will be resumed with the appointment of suitable personnel.

BUDGET DATA

10. --

11. --

12. BUDGET ACTIVITY: Research

13. IDENTIFY ANY COOPERATING UNITS:

Dept. of Physical Medicine and Rehabilitation, Cleft Palate Center, Dept. of Physiology of the Chicago Professional Colleges of the University of Illinois.

14. None

HONORS, AWARDS, AND PUBLICATIONS

15. --

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

Sleichter, C. G., Mandibular Distal Displacement: Its Relationship to Orthodontic Diagnosis and Treatment, The Angle Orthodontist, July 1955.

Sleichter, C. G.: A Simplified Head Positioner, J. of Prosthetic Dentistry, 5: 408-412, May 1955.

NOTE: Dr. Lloyd is Chief of the Dental Department in the Clinical Center.

R.P.C. - 1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

1. Dental Research
INSTITUTE

2. Clinical Investigations
BRANCH

3. --

4. --

5. NIDR-44(C)
SERIAL NO.

6. Maxillo-facial Prosthetics.
PROJECT TITLE

7. Ralph S. Lloyd
PRINCIPAL INVESTIGATOR

8. None
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION:

Objectives: To evaluate methods of treatment, designs of appliance and to observe the response of the patients.

Methods Employed: Different types of appliances are being constructed for individuals who have had destruction of tissues of the head and neck with surgery and patients afflicted with cleft palate. Improvement in esthetics, ability to ingest food (both solid and liquid), function of appliance and improvement in speech are observed. Speech therapy is given where advisable. The cephalometric measurements are utilized to help design these appliances, to determine the amount of freeway space, as well as to properly place the bulb in cleft palate devices.

Major Findings: The well being of the patients in cancer therapy and patients with cleft palate is greatly influenced by a properly made prosthetic device. The design of such a device is the basis of their success.

Significance to Dental Research: This is an area where very little work has been done and any contribution will be of great assistance in the treatment of these unfortunate patients.

Proposed Course of Project: To further investigate methods of construction, design of appliances and patient response to maxillo-facial prosthetic devices.

Analysis of NIH Program Activities

Budget Data Sheet

10. SERIAL NO. NIDR-44(C) 11. --

12. BUDGET ACTIVITY: Research 13. - 14. None

HONORS, AWARDS, AND PUBLICATIONS

15. - 17. None

NOTE: Dr. Lloyd is Chief of the Dental Department in the Clinical Center.

R.P.C. - 1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

1. Dental Research
INSTITUTE

2. Extramural Program

3. --

4. --

5. NIDR-45
SERIAL NO.

6. Extramural Program Summary Data.
PROJECT TITLE

7. Dale R. Lindsay
ACTING CHIEF

8. Supporting Staff

9. Summary Data

Research Grants: Grants are made to capable investigators in the field of Dental Research which are considered most likely to contribute to our knowledge of dental diseases and lead to advances in diagnostic, preventive measures and practical treatment. The awarding of grants to researchers in Dental Schools and other institutions has advanced far beyond the study of palliative measures and devices to fundamental studies in the causes and preventions of dental diseases.

The Dental Institute was appropriated $421,000 for grants for research projects. During the year 109 applications for grants were received for a sum of $1,029,184. Of those, 59 were approved by the National Advisory Dental Research Council totaling $494,701. Of the 59 approved only 29 grants could be awarded totaling $163,538. The remainder of the funds ($257,462) was allocated to pay the moral commitments.

Research Fellowships: This program is designed to develop scientifically qualified cancer investigators for research in the field of dental health.

A sum of $100,000 was made available for this activity for the fiscal year 1956. During the first six months of the fiscal year 10 regular fellowships were awarded totaling $35,766 and at present there are 5 fellowship applications pending amounting to $20,500.

A total of 18 part-time fellowships have been awarded to dental schools for 68 students for a total of $29,248.

In addition 7 supply grants have been made for a sum of $3,250.

10 - 17. None

R.P.C. - 1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

1. Dental Research
INSTITUTE

2. Office of the Director

3. --

4. --

5. NIDR-46
SERIAL NO.

6. Significant Contributions to the Administrative Operation of the Research Program
PROJECT TITLE

7. Francis A. Arnold, Jr.
NIDR Director

8. Supporting Staff
Others

9. Objectives and Significant Contributions

Objectives: The major objectives are to establish the policy, effective program development, and provide leadership for the various functions within the Institute. Also maintain an effective liaison with other Institutes, public and private organizations whose programs closely relate to our research functions.

Significant Contributions: At the beginning of the fiscal year a change was made in the responsibility for the expenditure of appropriated funds. For the first time appropriated funds were apportioned to the Laboratory and Branch Chiefs with full responsibility for the major part of the expenditures. With six months experience we feel that this procedure will provide a more economical method for expenditure of funds.

During the last few months we have reviewed position descriptions in our organizational structure for the purpose of bringing the positions up-to-date, promotions (particularly in the supervisory echelons), and better use of personnel.

Awards were received by two members of our staff in recognition of their research accomplishments. Dr. Frank J. McClure, Chief of the Laboratory of Oral and Biological Chemistry, received the Superior Service Award based on his continued meritorious contribution to the fields of nutrition and public Dentistry. Dr. David B. Scott, Chief of the Laboratory of Histology and Pathology, received the Arthur S. Flemming Award for developing new and useful techniques for using the electron microscope in Dental Research.

In the second quarter five hospital beds in the Clinical Center were allocated to the Institute. The activation of beds provides the Institute with an opportunity to study research problems heretofore never attempted in Dental Research. This will also afford us with an opportunity to make a team approach between our clinical and basic research programs.

10. --

11. --

12. BUDGET ACTIVITY: Administration

13 - 17. None

R.P.C. - 1
December 1955 Analysis of NIH Program Activities

Project Description Sheet

1. Dental Research
INSTITUTE

2. Office of Associate Director

3. --

4. --

5. NIDR-47
SERIAL NO.

6. Responsibilities of the Office of the Associate Director
PROJECT TITLE

7. Thomas J. Hill
Associate Director (Acting)

8. Supporting Staff
OTHERS

9. OBJECTIVES AND SIGNIFICANT CONTRIBUTIONS:

Objectives: To conduct, assist and direct laboratory and clinical research investigations at Bethesda and in the field as the research studies relate to causes, diagnosis, treatment and prevention of dental diseases. The most important objective of this office is to coordinate the research studies being conducted in the Clinical area with those in basic research laboratories.

Significant Contributions: Dr. Thomas J. Hill, serving as Associate Director, developed a proposed plan of operation for combined studies in clinical and laboratory research. This plan would be centered around a broad study of dental diseases and would involve the relationship of general health to oral disease and the influence of dental disease on general health. This plan is now being analyzed by the top staff to determine its feasibility.

10. --

11. --

12. BUDGET ACTIVITY: Research

13. - 17. None

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